EFFECTS OF PROLONGED USE OF EXTREMELY LOW-FAT DIET ON AN ADULT HUMAN SUBJECT [1]

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That some animal species are unable to synthesize linoleic and other highly unsaturated fatty acids and that these particular substances are essential for the proper nutrition of rats are observations which have been firmly established by the work of several investigators (Burr and Burr, '29; McAmis, Anderson and Mendel, '29; Evans and Lepkovsky, '32). Young animals placed on a diet extremely low in fat soon manifest several characteristic abnormalities, namely, retardation or complete cessation of growth, severe dandruff, scaliness of the feet and tail, hematuria and premature death. They also exhibit abnormal respiratory quotients. Administration of very small quantities of the essential unsaturated fatty acids or their esters results in the resumption of growth and in the rapid disappearance of all signs of the fat-deficiency disease. No such beneficial effects follow the ingestion of saturated fatty acids even in large amounts.

Very little is known regarding the dietary requirements of the human subject for unsaturated fatty acids. Mention can be made of but two published reports pertaining to the use of nearly fat-free diets by human subjects. Van Gröer ('19) maintained two infants on a diet extremely low in fat over a period of several months. Although not ideal, the growth

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of these infants was fairly good. However, both developed rickets, due to the lack of the fat-soluble vitamin D in the special diet. One of them also developed a generalized eczematous eruption of the skin. The latter complication was regarded with surprise by the author, because it occurred in spite of a regimen which was considered at the time to be ideal for the treatment of eczema. When re-examined some months after resumption of a complete diet, both infants were found to be essentially normal. Of three infants maintained on extremely low-fat diets by Holt and his associates ('35) one developed marked eczema. This subject experienced a clinical remission when fat was added to the diet, but again became eczematous when the low-fat diet was resumed. More recently two somewhat older infants were kept under observation at the University of Minnesota Hospital for 10 weeks on a diet extremely low in fat, though complete in other respects. During this period they developed no evidence of rickets and no skin manifestations other than repeated mild attacks of impetigo. However, studies of their blood lipids showed a moderate decrease in the degree of unsaturation of the serum fatty acids, similar to that found by Hansen and Burr ('33) and by Hansen and Brown ('37) in rats suffering from the fat-deficiency syndrome and that reported by Hansen ('37), by Faber and Roberts ('35) and by Schornstein ('37) in infants with severe eczema.

So far as we are able to ascertain, there is no record of any normal human adult's having been restricted for an extended period of time to a diet extremely low in fat. The present investigation was undertaken, therefore, to determine the effect on a healthy man of a diet practically devoid of fat. One of us volunteered to serve as the experimental subject. Preliminary examinations proved him to be an essentially normal adult, except for a slight elevation of blood pressure and a partial loss of one extremity. There was no indication that these disabilities would interfere with the value of the study. Observations were repeatedly recorded over a period of 6 months regarding the following factors: subjective feelings, objective clinical changes, body weight, basal metabolic

rate, respiratory quotients, carbohydrate tolerance, serum lipids, blood count and urinary findings.

MATERIALS AND METHODS

Special 'fat-free' diet

The diet was limited to sucrose, potato starch, baking powder, sodium chloride, ferric citrate, viosterol, carotene (vitamin A), orange juice, citric acid, anise oil, liquid petrolatum and milk practically freed of its fat. The daily protein intake was derived from 3 quarts of the specially defatted milk, taken as such, and the cottage cheese made from an additional quart of the same milk. Sucrose provided the bulk of the carbohydrate allowance but was supplemented by a biscuit made from potato starch, skimmed milk, baking powder, salt and mineral oil. The mineral oil was added to serve as shortening and to prevent constipation. Daily supplements of 10 mg. of ferric citrate, 2.5 mg. of carotene, 0.02 cc. concentrated viosterol in oil (8000 U.S.P. units vitamin D) and the juice from one-half of a large orange were given to insure an adequate supply of iron and of vitamins. That the diet was of the extremely 'low-fat,' rather than the 'fat-free,' type was recognized when the experiment was planned, but experience with crude diets (Burr and Brown, unpublished data) in studies on the rat had shown this to be satisfactory for our purpose. The chief source of fat in the diet was the skimmed milk. Periodic analyses of this milk as specially prepared showed it to have an average fatty acid content of less than 0.08%. This type of fat (butterfat) has been found to be of such low protective or curative value for rats on a fat deficient regimen that the 2 gm. contained in the daily diet of our subject was not considered sufficient to affect seriously the experiment. As potato starch contains only minute traces of fat, the small amount fed was likewise considered unimportant. A uniform consumption of food, providing 2500 calories daily, was maintained throughout the experiment. The sugar was taken in the form of a syrup with citric acid or anise added for flavoring. Frequent small meals were

found to be most satisfactory. The syrup was taken at hourly intervals from the time of rising until the time of the evening meal. The orange juice was taken at bed time. Cottage cheese with the biscuit and milk comprised the evening meal. It was calculated that a rat, consuming this diet in proportion to its caloric requirements, would receive less than 4 mg. of fat daily. This amount would be equivalent to approximately 1 drop of oil per week. In order to check the diet as to its effect on experimental animals, a group of twelve rats was placed on a mixture of potato starch and sucrose, supplemented by the specially skimmed milk given ad libitum. They

TABLE 1

Average growth of test rats maintained on the experimental diet as compared with that of control rats on normal stock diet

AGE	RATS ON LOW-FAT DIET	RATS ON NORMAL DIET
days	*Weight in grams*	*Weight in grams*
28	52	49
56	106	124
84	140	164
112	155	190
140	159	195
168	166	200
196	167	210
224	167	215
252	160	218

developed all of the characteristic symptoms of the fat deficiency syndrome in approximately the same time and with the same degree of severity as recorded for rats on Burr's diet 550B. The retardation in their growth is shown in table 1 in which their average weights at different ages are compared with those of control animals on a regular stock diet.

Methods used

The serum lipids were determined on fasting blood specimens by the oxidative methods of Bloor ('28). The iodine absorption capacity of the serum was determined by the pyridine sulphate dibromide method (Yasuda, '31). The linoleic

and arachidonic acids were determined by the technic described by Brown and Hansen ('37). One set of determinations of the serum lipids was made by the method of Wilson and Hansen ('36) 2 months after the normal diet was resumed. Standard methods were used for counting the erythrocytes and leucocytes of the blood and for examining the urine and stools. The same scale and mercury manometer were used throughout for determining the body weight and the blood pressure. The basal metabolism was determined by the ordinary indirect technic, and the respiratory quotients by the closed chamber method. Routine urinary and physical examinations were made at weekly intervals.

RESULTS

The results of the study are presented below.

Clinical data

The subject remained clinically well throughout the entire period of observation, not having even a common cold. There was never any itching of the skin nor pain. At no time did any of the food ingredients [2] become distasteful. One of the most noticeable subjective effects of the diet was the marked absence of fatigue. The somewhat tired feeling usually experienced after a day's work in the laboratory disappeared within a few days from institution of the diet. From childhood the subject had suffered from frequent attacks of migraine. These had been occurring at intervals of 7 or 8 days immediately before the present experiment was begun. After being 6 weeks on the diet, he observed that these periodic attacks of headache had subsided completely. Strangely enough, they have never recurred.

The complete physical examinations, made 1 week apart just before the experiment was begun, revealed no definite abnormality other then a mild degree of arterial hypertension.

[2] The authors wish to express their gratitude to Margaret Day Hansen for her invaluable assistance in preparing the fat-free diet.

It was particularly observed that the skin and mucous membranes were clear and soft. The only demonstrable physical changes resulting from the diet, as determined by regular weekly examinations were a moderate loss of body weight and a decrease in blood pressure. Blood pressure readings, taken when the subject was on a normal diet, varied between 140 to 150 mm. of mercury systolic and 95 to 100 diastolic, values which were regarded as being definitely higher than normal. There was a distinct decrease in the blood pressure after institution of the fat-free diet, the minimum values being obtained 4 to 5 months after the diet was started. Readings at that time remained quite consistently around 130 mm. of mercury systolic and 85 to 88 diastolic. Several months after the low-fat diet had been discontinued, the blood pressure had again risen to its former level.

There was a gradual decrease in weight during the first 3 months from 152 pounds (69.1 kg.) to 138 pounds (62.7 kg.). The weight thereafter remained about the same for nearly 3 months, that is, until the special diet was discontinued. This decrease in body weight in spite of a supposedly adequate caloric intake is of special interest. Unfortunately, however, the exact composition of the weight loss could not be determined.

Energy metabolism

The basal metabolic rate before and several months after the experimental period varied between —9 and —12% on four occasions, whereas it was found to be —2% just before the low-fat diet was discontinued. The respiratory quotients showed a distinct alteration. One of the most striking effects of low-fat diet on the rat is the rise in respiratory quotient after a meal. A similar tendency was found in the case of our human subject. During the sixth month of the experimental diet the following respiratory quotients were obtained: 1.03, 1.11 and 1.14. To attain these quotients it was necessary to starve the subject overnight and then give him a liberal supply (over 2000 calories) of the sugar-milk diet within the

course of 2 hours. Two hours later the maximum quotient was reached. Using the same technic the highest quotients reached before and after the low-fat experimental period were 0.99 and 0.97. It would seem, therefore, that in this respect the human subject reacts to a low-fat diet in the same way that the rat does.

Blood constituents

The data on the serum lipids are presented in table 2. It is apparent that the iodine number of the total fatty acids was definitely lowered as a result of the fat-poor diet. The total drop in iodine number from an average of several preliminary determinations was approximately 30. There was

TABLE 2

Serum lipids of experimental subject on normal diet and on low-fat diet

DATE	IODINE ABSORPTION	CHOLESTEROL	T.F.A.	TOTAL LIPIDS	I.N. OF T.F.A.
Normal control diet					
	mg. per 100 cc.	*mg. %*	*mg. %*	*mg. %*	
11–23–33	664	252	402	654	124
12– 1–33	775	252	504	756	121
Low-fat diet					
1–26–34	753	298	607	899	92
2–10–34	799	228	705	933	92
5–20–34	643	206	538	746	94

no essential change in the serum cholesterol as a result of the change in diet. However, the total fatty acid values increased somewhat, especially in the early part of the special dietary period. A determination of the serum lipids by the microgravimetric technic of Wilson and Hansen ('36) 2 months after resumption of a normal diet gave essentially normal values as follows: unsaponifiable fraction, 305.0 mg. per cent; total fatty acids (saponifiable fraction), 449.0 mg. per cent; average molecular weight of total fatty acids, 293; iodine number, 97, with 1.23 double bonds per molecule of fatty acid. This value for the iodine number of the total fatty acids is within the normal range as determined by this method.

The finding of a fall in the degree of unsaturation of the serum lipids on a low-fat regimen tends to confirm for the human subject the observations of several investigators regarding various other species of animals. Hansen and Burr ('33), Williams and Maynard ('34), and Hansen, Wilson and Williams ('36) found the iodine numbers of the serum lipids to be definitely decreased by low-fat diets in rats, goats and dogs, respectively. Hansen and Brown ('37) observed that small quantities of unsaturated fatty acids caused a regression of the fat deficiency symptoms well in advance of an appreciable change in the iodine number of the serum fatty acids.

Before the special diet was given to our subject, the values for both arachidonic acid and linoleic acid of the serum were similar to those found in other normal subjects by Brown and

TABLE 3

Arachidonic and linoleic acid content of serum of experimental subject on normal diet and on low-fat diet

DIET	ARACHIDONIC ACID	LINOLEIC ACID
	per cent of T.F.A.	*per cent of T.F.A.*
Normal (11–30–33)	3.2	5.7
Low-fat (for 6 months)	1.87	3.2

Hansen ('37). Tängl ('30) reported normal ox blood to contain arachidonic acid to the extent of 5 to 10% of the total fatty acids. We have been unable to find any other work dealing with this phase of the blood lipid picture. The finding, as shown in table 3, of a marked drop in the content of both arachidonic and linoleic acids in the case of our subject confirms the previously mentioned observation of a drop in the iodine number of the total fatty acids of the serum as a result of the low-fat regimen. No other information is available concerning the effect of diet on these individual highly unsaturated fatty acids of the blood. It may be mentioned that Brown and Hansen ('37) found the content of both arachidonic acid and linoleic acid to be significantly decreased in active infantile eczema, as determined on pooled lipid extracts from many samples of blood taken from patients with

this disorder. The values found for the eczematous infants were of approximately the same magnitude as those found in the case of this subject after he had remained for some months on the low-fat diet. The change in the serum lipids in eczema, however, has not been related to use of a low-fat diet in the subjects studied.

Other blood constituents showed no significant change. The fasting blood glucose remained normal and the sugar tolerance curve was found to be normal on two occasions toward the end of the study. The increase in blood sugar following the oral administration of glucose was accompanied by the normal phenomenon of a decrease in the level of the inorganic phosphorus of the plasma. Fasting values of the inorganic phosphorus on two occasions during the experimental period were 3.43 mg. and 2.64 mg. phosphorus per 100 cc. of plasma, respectively. Six months after the resumption of the normal diet, the fasting inorganic phosphorus of the plasma was 4.2 mg. per cent. The serum calcium level was uninfluenced by the special diet, remaining throughout the entire period around 10.0 mg. per cent. The total proteins of the serum were found to be 6.98 gm. per cent while on the normal diet and 8.06 gm. per cent on the low-fat diet. Corresponding values for the non-protein nitrogen of the serum were 29.4 and 32.1 mg. per cent on these occasions.

No definite morphological change occurred in the blood, other than a tendency to leucopenia. The hemoglobin remained between 90 and 95% during the low-fat dietary period with an average red cell count of 4.6 million per cubic millimeter. The leucocyte count was usually 4000 to 5000 per cubic millimeter on the low-fat regimen, while at other times it was 5000 to 6000. On one occasion during the experimental period, it fell to 2000 cells per cubic millimeter. The differential count always remained essentially normal as regards the distribution of the various cell types. The hematologist was unable to detect any abnormality in the morphology of the cells during the experimental period.

Urine examinations

Since the occurrence of hematuria is a distinct characteristic of the fat-deficiency syndrome in rats and is a serious prognostic omen, the urinary sediment was examined very closely, especially for blood. Frequent routine examinations of the urine failed to show any abnormality as to the presence of protein, sugar, abnormal sediment, diacetic acid or acetone.

DISCUSSION

That an adult human being can subsist on a diet containing a total of less than 2 gm. of fat per day (0.03 gm. per kilogram of body weight) for at least 6 months without demonstrable harm is somewhat remarkable. There is no assurance, however, that this subject or any other could be maintained indefinitely in a state of good health on such a regimen. The young rats, that developed typical signs of fat-deficiency diseases on this diet, lived a very much larger percentage of their life span on the regimen before showing abnormalities than that represented by 6 months in the span of a human life. Age or maturity is undoubtedly an important factor influencing the susceptibility to this as to other types of dietary deficiency also, the mature subject usually being much more resistant than the rapidly growing subject.

The mere fact that our subject at the end of the 6-month period showed changes in the serum lipids (decrease in the unsaturated fatty acids) and respiratory quotients, which were the same as those shown by rats suffering from unsaturated fatty acid deficiency, indicates that the response is qualitatively the same in the two species. While the human subject, like the rat can synthesize some fats, he apparently cannot fabricate the highly unsaturated fatty acids in sufficient amounts to maintain their concentration in the serum at levels found to exist when they are available in the diet. In all probability, therefore, the human subject, as well as the rat, would ultimately show clinical evidence of this deficiency, if the restricted diet were continued over a much longer period of time.

The small but definite decrease in the subject's blood pressure was probably due to loss in body weight, rather than to a specific effect of the low-fat diet. Disappearance of long experienced, periodic attacks of headache cannot be explained on any known basis, unless it be assumed that this symptom complex had been independent in part upon the moderate degree of arterial hypertension that existed before the special diet was used. That blood pressure was not the sole factor in the causation of the headache, however, is suggested by the fact that the symptom had failed to recur many months after resumption of a normal diet and return of the blood pressure to its previous degree of elevation.

The most interesting subjective effect of the 'fat-free' regimen was the definite disappearance of a feeling of fatigue at the end of the day's work. This experience was diametrically opposite that reported by Murlin and co-workers ('33) from the use of diets extremely high in fat and low in carbohydrate. It is impossible to say, whether this difference in feeling is simply a subjective representation of a difference in energy availability from the two diets, or, whether the sedative or slightly toxic effect of the ketone bodies accumulating in the tissues as a result of the latter type of diet is responsible for this effect.

That the subject of this experiment showed no increase in susceptibility to acute infections as a result of his extremely low fat intake is worthy of special note in light of recent observations regarding the relationship of fat metabolism to infection. For instance it has been pointed out repeatedly that a high-fat, low-carbohydrate dietary appears to reduce susceptibility to acute respiratory disorders (Spiesman and Arnold, '37; Hoelzel, '37). In fairly severe acute infections all of the blood lipids are greatly reduced and the iodine number of the serum fatty acids is decreased (Achard, Grigant, Leblanc and David, '28; McQuarrie and Stoesser, '35; Stoesser, '35). A possible explanation for our subject's apparent failure to manifest an increased susceptibility to infections may be found in the fact that his total serum lipids

were maintained at normal levels. Obviously the point does not deserve to be stressed because the factor of exposure to outside sources of infection was not adequately controlled.

SUMMARY AND CONCLUSIONS

1. An adult human subject was maintained for a period of 6 months on a nearly fat-free diet (less than 0.03 gm. of fat per kilogram of body weight per day) without demonstrable harm.

2. Young rats placed on the same diet developed the typical fat deficiency syndrome.

3. The only objective clinical effects of the diet on the adult human subject were a) a gradual loss of body weight during the first 3 months from 69 to 62 kg. and b) a coincident reduction to normal of a slightly elevated arterial blood pressure.

4. Subjectively the most striking effects were, a) disappearance of a previously experienced feeling of fatigue at the end of the day's work and b) disappearance of recurrent attacks of migraine-like headache from which the subject had suffered for some years prior to the time of the present experiment.

5. The respiratory quotient following a large meal of the experimental diet rose to 1.04, 1.11 and finally to 1.14 on different occasions during the sixth month of the low-fat dietary period; whereas under identical conditions before and after the period, it never rose above 0.97 and 0.99 respectively. In this respect the effect of the diet on the human subject is the same as that in the rat.

6. Repeated determinations of the basal metabolic rate before and after the experimental period gave values varying between —9 and —12%, whereas during the sixth month of the low-fat dietary period it was —2.

7. The iodine number of the total fatty acids of the serum from fasting blood specimens was decreased from an average of 123 before the special dietary period to an average of 93 during the period. At the same time the linoleic acid of the serum was found to have fallen from 5.7 to 3.2% of the total fatty acids and arachidonic acid from 3.2 to 1.8%. These

changes in the serum fatty acids are similar to those found in rats suffering from the use of diets deficient in unsaturated fatty acids (Hansen and Burr, '33) and in human infants suffering from eczema (Hansen, '33, '37; Brown and Hansen, '37).

8. Decrease in the unsaturated fatty acids as a result of the low-fat regimen indicates the probability that even the normal adult human subject, like the rat, is unable to fabricate the highly unsaturated fatty acids, which should, therefore, be provided in the diet.

9. In the light of this latter observation, it cannot be assumed that the human subject could subsist indefinitely on a diet completely devoid of the unsaturated fatty acids.

LITERATURE CITED

ACHARD, C., A. GRIGAUT, A. LEBLANC AND M. DAVID 1928 L'equilibre lipoidique du serum sanguin dans les maladies aiguës. J. physiol. et path. gen., T. 26, p. 415.

BLOOR, W. R. 1928 The determination of small amounts of lipid in blood plasma. J. Biol. Chem., vol. 77, p. 53.

BROWN, W. R., AND ARILD E. HANSEN 1937 Arachidonic and linolic acid of the serum in normal and eczematous human subjects. Proc. Soc. Exp. Biol. and Med., vol. 36, p. 113.

BURR, G. O., AND W. R. BROWN 1938 Unpublished data. University of Minnesota.

BURR, G. O., AND M. M. BURR 1929 A new dietary disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., vol. 82, p. 345.

EVANS, H. M., AND S. LEPKOVSKY 1932 Vital need of the body for certain unsaturated fatty acids. III. Inability of the rat organism to synthesize the essential unsaturated fatty acids. J. Biol. Chem., vol. 99, p. 231.

FABER, H. K., AND D. B. ROBERTS 1935 Studies of serum lipids in infantile allergic eczema with special reference to saturation of fatty acids. J. Pediatrics, vol. 6, p. 490.

HANSEN, ARILD E. 1933 Study of iodine number of serum fatty acids in infantile eczema. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 1198.

———— 1937 Serum lipids in eczema and in other pathologic conditions. Am. J. Dis. Child., vol. 53, p. 933.

HANSEN, ARILD E., AND W. R. BROWN 1937 The effect of low fat diets on serum lipids of rats. J. Nutrition, vol. 13, p. 351.

HANSEN, ARILD E., AND G. O. BURR 1933 Iodine numbers of serum lipids in rats fed on fat-free diets. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 1201.

HANSEN, ARILD E., WM. R. WILSON AND H. H. WILLIAMS 1936 Serum lipid changes in relation to the intermediary metabolism of fat. J. Biol. Chem., vol. 114, p. 209.

HOELZEL, F. 1937 Diet and resistance to colds. Science, vol. 86, p. 399.

HOLT, L. E., JR., H. C. TIDWELL, C. M. KIRK, D. M. CROSS AND S. NEALE 1935 Studies in fat metabolism. I. Fat absorption in normal infants. J. Pediatrics, vol. 6, p. 427.

MCAMIS, A. J., W. E. ANDERSON AND L. B. MENDEL 1929 Growth of rats on fat-free diets. J. Biol. Chem., vol. 82, p. 247.

MCQUARRIE, I., AND A. V. STOESSER 1935 Influence of acute infection and artificial fever of plasma lipids. Am. J. Dis. Child., vol. 49, p. 658.

MURLIN, J. R., E. E. HAWLEY AND C. W. JOHNSON 1933 The possibility of gluconeogenesis from fat. II. The effect of high fat diets on the respiratory metabolism and ketosis in man. J. Nutrition, vol. 6, p. 523.

SCHORNSTEIN, L. 1937 Über den Gehalt der Serumfette an ungesättigten Fettsauren beim Säugelingsekzem. Ztsch. f. Kinderheilk., Bd. 59, S. 52.

SPIESMAN, I. G., AND L. ARNOLD 1937 Host susceptibility to common colds. Am. J. Digest. Dis. and Nutrition, vol. 4, p. 447.

STOESSER, A. V. 1935 Effect of acute infection on iodine number of serum fatty acids. Proc. Soc. Exp. Biol. and Med., vol. 32, p. 1326.

TÄNGL, H. 1930 Mikromethode zur Bestimmung von vierfach ungesättigten Fettsauren. Biochem. Ztsch., Bd. 226, S. 180.

VON GRÖER, F. 1919 Zur Frage der praktischen Bedeutung des Nährwertbegriffes nebst einigen Bemerkungen über das Fettminimum des menschlichen Säuglings. Biochem. Ztsch., Bd. 97, S. 311.

WILLIAMS, H. H., AND L. A. MAYNARD 1934 The effects of specific dietary fats on the blood lipids of lactating goats. J. Dairy Science, vol. 17, p. 223.

WILSON, W. R., AND ARILD E. HANSEN 1936 Study of the serum lipids by a micrograviметric technique. J. Biol. Chem., vol. 112, p. 457.

YASUDA, M. 1931 The determination of the iodine number of lipids. J. Biol. Chem., vol. 94, p. 401.

THE EFFECT UPON HEMATOPOIESIS OF VARIATIONS IN THE DIETARY LEVELS OF CALCIUM, PHOSPHORUS, IRON AND VITAMIN D [1, 2]

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TWO FIGURES

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Since the early part of this century when von Wendt ('05) and Sherman ('07) suggested that calcium exercises a sparing action upon iron, it has been rather generally assumed that generous amounts of dietary calcium tend to promote iron utilization (Davidson, Fullerton, Howie, Croll, Orr and Godden, '33). However the suggested relationship has never been clarified. Orten, Smith and Mendel ('36) studied the relation of calcium and iron to hematopoiesis in rats restricted to a low-mineral ration. The observed polycythemia and concurrent mild anemia, characteristic of rats maintained on the basal ration, was prevented by the addition of calcium carbonate alone to the diet. Ferric chloride alone tended to produce a normal blood picture but the results were not as favorable as with calcium. They concluded "that the hematological abnormalities which occur in rats as a result of the feeding of the mineral-deficient ration are due chiefly, if not entirely, to a lack of calcium and/or iron."

[1] Presented by Harold J. Stein to the faculty of the School of Hygiene and Public Health, Johns Hopkins University, in partial fulfillment of the requirements for the degree of Doctor of Science in Hygiene.

[2] Part of the data reported here were presented to the thirty-second annual meeting of the American Society of Biological Chemists, Baltimore, March 31 to April 2, 1938.

The idea tends to find veiled expression in their paper that calcium has some unique role in hematopoiesis. For example, it is stated that the data "may be interpreted as indicating that the response to iron was dependent upon the presence of some other substance. In view of the invariably favorable responses to calcium supplementation, it appears likely that the other substance is calcium itself . . . ." Although this view appears to dominate their interpretation of the data, they did suggest that phosphorus or perhaps the Ca : P ratio may be involved in the syndrome. But the suggestion was neither emphasized nor tested.

Apparently the Journal of the American Medical Association (American Medical Association, '37) has accepted the implication of Orten et al. that calcium plays a special role in blood formation. In an editorial given exclusively to comment on the work it is significant that the possible importance of phosphorus or of the Ca : P ratio was not mentioned.

An interpretation of the data of Orten and associates should include consideration of the fact that their basal ration contained a relatively large excess of phosphorus in comparison to the content of calcium, iron and other minerals. The analyses of Smith and Smith ('34) expressed in terms of the molecular ratios, taking calcium as unity, were: Ca, 1.0; Mg, 1.4; Na, 6.8; Cl, 11.1; K, 35.2 and P, 72.5. Accordingly a reasonable explanation of the favorable effects of calcium carbonate is that the calcium combined with the relative excess of phosphorus as calcium phosphate, thus permitting the small amount of iron to be used in blood formation rather than being excreted by the free phosphate. Since Waltner ('27), Cox, Dodds, Wigman and Murphy ('31), Brock and Diamond ('34) and Deobald and Elvehjem ('35) have produced marked degrees of phosphorus deficiency in animals by the addition of soluble iron salts to a diet otherwise adequate, this explanation is indeed plausible.

Obviously a clear understanding of the relationship of calcium to iron in hematopoiesis is of importance and experiments which contribute to its elucidation are timely and

significant. Accordingly we have investigated the problem to determine a) whether calcium has a unique role in hematopoiesis; b) the general relationships between hematopoiesis and the levels of calcium and phosphorus in the diet; and c) the effect of vitamin D deficiency on blood formation when the levels of calcium and iron are abnormal.

EXPERIMENTAL

Basal ration for first series (I). In the first series the basal ration and vitamin supplements described by Orten, Smith and Mendel were used exactly as employed by those workers, including controlled feeding in which the caloric intake was the same for each animal. The basal ration was: Casein (acid washed), 18; dextrin, 55; and hydrogenated fat (Crisco), 27. The vitamin supplements per rat daily were: Wheat germ extract, 1 cc. = 2 gm.; wheat germ oil, 2 drops; cod liver oil, 4 drops; and dried yeast, 200 mg.

Basal rations for second series (II). The composition of each ration was:

	18	*18 D*
Casein (acid washed)	18.00	18.00
Sucrose	66.06	66.06
Salts no. 3 (Ca, P, and Fe-free)	2.89	2.89
Hydrogenated fat (Crisco)	12.00	12.00
Carotene in corn oil (3:1000)	0.30	0.30
Vitamin B complex	0.75	0.75
Viosterol	None	15 drops per kg.

The composition of salts no. 3 was: NaCl, 39.98; KCl, 18.31; $KHCO_3$, 27.43; $MgSO_4 \times H_2O$, 12.98; $CuSO_4 \cdot 5H_2O$, 0.44; $MnSO_4 \cdot 2H_2O$, 0.14; $ZnSO_4 \cdot 7H_2O$, 0.69; and KI, 0.03. In terms of the amount of basal rations fed per rat daily (5 gm.) the calcium, phosphorus, and iron contents were respectively: 0.3, 5.2, and 0.023 mg. The amount of phosphorus could have been greatly reduced by the use of purified edestin instead of casein, but for the purposes of this investigation casein was quite satisfactory.

Vitamin B complex. This preparation, made from dilute alcoholic extracts of yeast and wheat germ, was devised by one of us (H.G.D.) for use in the study of phosphorus de-

ficiency in rats. Since it is low in phosphorus, iron and calcium it was regarded as satisfactory for the present studies. The details of its preparation are given here since they have not been published elsewhere.

Three hundred grams of yeast were added to 2115 cc. of 35% ethyl alcohol and stirred for approximately 1 hour. The extract was then centrifuged and the supernatant liquid concentrated in vacuo to about one-quarter of its original volume. The concentrate was placed in a refrigerator overnight and then filtered. The volume of the filtrate was adjusted to approximately 550 cc., and 12 gm. of pulverized $Ca(OH)_2$, C.P., were slowly added. The solution was stirred mechanically for ½ hour. This was followed by the slow addition of 640 cc. of 95% ethyl alcohol. About 45 minutes were required for this and stirring was continued without cessation. The mixture was immediately centrifuged to remove the insoluble calcium salts and other precipitated material as well as the excess of $Ca(OH)_2$. The clear supernatant fluid was poured off and the reaction was adjusted to pH4-5 using concentrated HCl.

The wheat germ concentrate was prepared by adding 400 gm. of wheat germ to 2820 cc. of 35% ethyl alcohol. This was stirred for about 1 hour. The extract was centrifuged and concentrated as above to approximately one-quarter of the original volume. The concentrate was left overnight in the refrigerator and then filtered. Following this the volume of the filtrate was adjusted to about 700 cc., and 16 gm. of $Ca(OH)_2$ were added with stirring for ½ hour. Then 800 cc. of 95% ethyl alcohol were added slowly, stirring as in the preparation of the yeast concentrate. The supernatant fluid was obtained by centrifuging and acidifying as described above.

Following this the two acidified extracts (yeast and wheat germ) were combined and concentrated in vacuo to a volume of approximately 250 cc. This was fortified by the addition of 40 mg. of nicotinic acid. The preparation, containing an adequate amount of all rat factors of the vitamin B complex as ascertained by numerous assays in this laboratory, was poured onto 3970 gm. of the basal ration (18 or 18 D), and thoroughly mixed with it. Then the mixture was dried by means of an electric fan, pulverized with a meat grinder and stored in the refrigerator until used. Fresh diets were made up every 2 or 3 weeks.

Cages. All animals were kept in individual galvanized metal cages. The screens were fairly effective in preventing access to the excreta. They were washed twice weekly. Glass or porcelain feeding cups were used exclusively.

Hematological methods. Blood samples were obtained according to a uniform procedure which involved clipping the end of the tail with sharp scissors but without exciting the animal or causing appreciable loss of blood. Hemoglobin was determined by the Newcomer method using an accurately standardized color filter. The erythrocyte counts were made in the standard manner using Levy-Hausser counting chambers. Samples were counted in duplicate and values failing to check within 100,000 to 130,000 per cubic millimeter were discarded. The pipettes, counting chambers, and cover slips were certified by the U. S. Bureau of Standards. Standard statistical methods were used in the calculation and interpretation of the data.

Procedure for first series (I). A total of sixty stock male rats weighing 55 to 60 gm. and a like number of females weighing 45 to 50 gm. were weighed and continued on the stock colony diet 12 or 14 days. Out of these groups fifty males and fifty females were selected whose average weights were closest to 120 gm. and 100 gm. respectively. These animals were divided into ten different groups with the sexes evenly distributed, viz., five males and five females in each group. One group was restricted to the basal ration plus vitamin supplements, as described above (diet I), and the remaining groups were restricted to the same basal ration and vitamin regimen plus mineral supplements as indicated:

Diet II.	Same as diet I plus	1.4 mg.	Fe as $FeCl_3 \cdot 6H_2O$ per rat daily
Diet III.	Same as diet I plus	50.0 mg.	Ca as $CaCO_3$ per rat daily
Diet IV.	Same as diet I plus	0.9 mg.	Be as $BeCO_3 \cdot 4H_2O$ per rat daily
Diet V.	Same as diet I plus	6.0 mg.	Fe as $FeCl_3 \cdot 6H_2O$ per rat daily
Diet VI.	Same as diet I plus	50.0 mg.	Ca as $CaHPO_4$ per rat daily
Diet VII.	Same as diet I plus	6.0 mg.	Fe as $FePO_4 \cdot 4H_2O$ per rat daily
Diet VIII.	Same as diet I plus	1.4 mg. Fe as $FeCl_3 \cdot 6H_2O$ and 0.9 mg. Be as $BeCO_3 \cdot 4H_2O$ per rat daily	
Diet IX.	Same as diet I plus	6.0 mg. Fe as $FePO_4 \cdot 4H_2O$ and 0.9 mg. Be as $BeCO_3 \cdot 4H_2O$ per rat daily	
Diet X.	Same as diet I plus	233.0 mg.	Salts no. 51 (McCollum) per rat daily

Since Guyatt, Kay and Branion ('33), as well as others, had shown that beryllium carbonate binds excess phosphorus, thus preventing its utilization, the use of beryllium carbonate in certain of the rations in understandable. Salts no. 51 (McCollum) has the composition: $CaCO_3$, 24.58; KCl, 16.40; NaCl, 8.20; $NaHCO_3$, 11.48; MgO, 3.28; ferric citrate, 8.20; and KH_2PO_4, 27.86.

Procedure for second series (II). A total of forty stock males weighing 45 to 50 gm. and a like number of females weighing 35 to 40 gm. were weighed and continued on the stock colony diet 10 to 14 days. Out of these groups thirty-five males and thirty-five females were selected whose average weights were closest to 93 and 83 gm. respectively. These animals were divided into seven different groups with the sexes evenly divided as in the case of series I. Five groups were fed basal ration 18D (containing vitamin D) plus mineral supplements and the other two groups were restricted to basal ration 18 (without vitamin D) plus mineral supplements as indicated.

Diet	XIX.	18D	plus	19.5	mg.	Ca,	19.5	mg. P and	2.3	mg. Fe per rat daily.
Diet	XX.	18D	plus	19.5	mg.	Ca,	58.5	mg. P and	2.3	mg. Fe per rat daily.
Diet	XXI.	18D	plus	3.9	mg.	Ca,	19.5	mg. P and	2.3	mg. Fe per rat daily.
Diet	XXII.	18D	plus	19.5	mg.	Ca,	19.5	mg. P and	0.23	mg. Fe per rat daily.
Diet	XXIII.	18D	plus	3.9	mg.	Ca,	19.5	mg. P and	0.23	mg. Fe per rat daily.
Diet	XXIV.	18	plus	3.9	mg.	Ca,	19.5	mg. P and	2.3	mg. Fe per rat daily.
Diet	XXV.	18	plus	3.9	mg.	Ca,	19.5	mg. P and	0.23	mg. Fe per rat daily.

The calcium was furnished as C.P. $CaCO_3$, phosphorus as C.P. $NH_4H_2PO_4$, and iron as C.P. $FeCl_3 \cdot 6H_2O$. The feeding was controlled, as in series I, so that all groups had the same caloric intake. Each animal received 5 gm. daily of the appropriate basal ration in addition to the mineral supplements which were mixed with the rations. The care of these animals, hematological determinations, etc., were identical with those used in series I, except that the experimental period lasted 8 weeks.

RESULTS

First series (I). The average biweekly erythrocyte counts and hemoglobin values for each group of animals on diets I to X are given in figure 1. The rise in values during the first few weeks are attributable to age changes. Diets I (without mineral supplements), II (1.4 mg. Fe as ferric chloride), and III (50.0 mg. Ca as $CaCO_3$) are identical with those used by Orten, Smith and Mendel and the hematological results are in essential agreement. Without mineral supplements there was a polycythemia and mild anemia which became increasingly abnormal as the deficiency progressed. The addition of small amounts of ferric chloride only partially prevented the polycythemia and anemia, but calcium carbonate produced an entirely normal blood picture.

The other diets in this series were planned to elucidate the nature of the calcium and iron action. Diet IV (0.9 mg. Be as beryllium carbonate) produced a normal blood picture scarcely distinguishable from that with diet III, although neither calcium nor iron was added. With diet V (6.0 mg. Fe as ferric chloride) the hematological results were entirely normal. Since this diet was not supplemented with calcium the Ca: P ratio was the same as in diets I and II. Although diet VI contained the same amount of calcium as diet III the element was furnished as dicalcium phosphate. Animals restricted to this ration developed a degree of anemia and polycythemia equal to that of animals without any mineral supplements (diet I). Similarly rats restricted to diet VII (6.0 mg. Fe as ferric phosphate) showed a considerable degree of polycythemia and anemia, the values being only slightly less abnormal than those of animals on diet I. However, the iron content was identical with that of diet V.

Diets VIII and IX contribute but little to the elucidation of the role of calcium in hematopoiesis. The first contained the same amounts of iron and beryllium as diets II and IV respectively, and in the forms employed in those diets. The second contained iron as in diet VII and beryllium as in diet IV. Since the hematological values were essentially normal

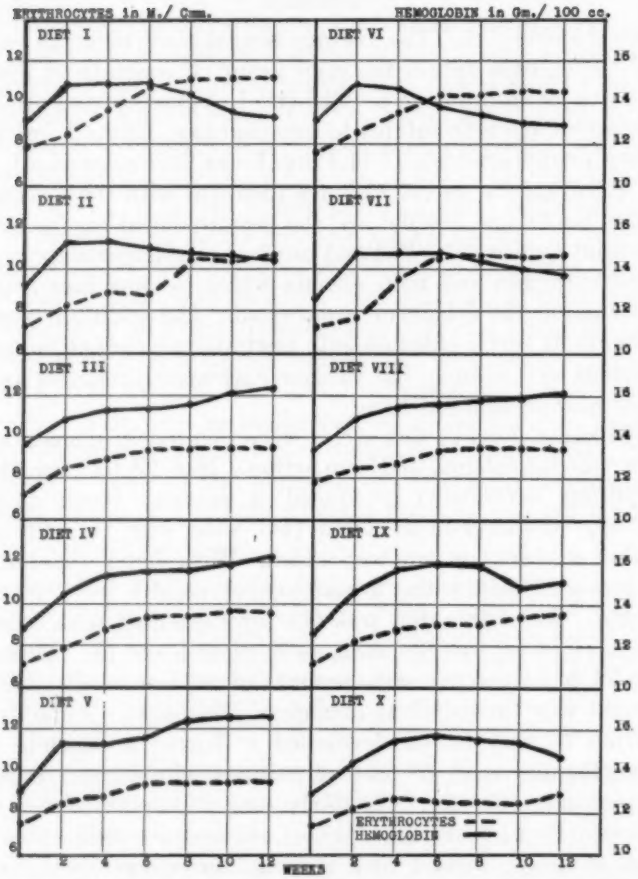

Fig. 1 Average erthrocyte and hemoglobin content of blood from rats maintained on a low-mineral ration supplemented per rat daily as follows: I, no supplement; II, 1.4 mg. Fe as ferric chloride; III, 50 mg. Ca as calcium carbonate; IV, 0.9 mg. Be as beryllium carbonate; V, 6.0 mg. Fe as ferric chloride; VI, 50 mg. Ca as dicalcium phosphate; VII, 6.0 mg. Fe as ferric phosphate; VIII, 1.4 mg. Fe as ferric chloride and 0.9 mg. Be as beryllium carbonate; IX, 6.0 mg. Fe as ferric phosphate and 0.9 mg. Be as beryllium carbonate; and X, 233 mg. McCollum salts no. 51.

with both diets it is indicated that the small amount of iron used to supplement diet VIII was not necessary to produce adequate hematopoiesis and that ferric phosphate has no deleterious effect when provision is made to bind the relative excess of phosphorus in the basal ration. Diet X (McCollum salts no. 51) was employed simply to provide comparative data from animals on an approximately normal diet but with restricted caloric intake. The results indicate that caloric restriction has no significant effect on blood formation.

Second series (II). The average biweekly erythrocyte counts and hemoglobin values for each group of animals on diets XIX to XXV are given in figure 2. It is evident that the results with diet XIX (presumably adequate levels of Ca, P and Fe) are quite normal. The average erythrocyte count at the end of the eighth week was 8.68 ± 0.21 million per cubic millimeter and the corresponding average hemoglobin value was 15.51 ± 0.32 gm. per 100 cc. of blood. Diet XX (normal Ca and Fe but an excess of P) caused definite anemia and polycythemia. In this case the average red blood cell count at the end of the eighth week was 9.49 ± 0.14 million per cubic millimeter. The concentration of hemoglobin was 12.52 ± 0.32 gm. per 100 cc. of blood. The occurrence of an increase of erythrocytes is indeed interesting since presumably the only abnormality of the diet was the high level of phosphorus. That failure of iron utilization is a consequence of excess dietary phosphorus, whether absolute or relative, is further shown by the data from diet XXI (normal P and Fe but low Ca). Here the erythrocyte picture was similar to that in diet XX but the hemoglobin level was slower in reaching the terminal value which obtained with diet XX.

The effect of the dietary iron content on blood formation with a normal Ca : P ratio, as compared with an abnormal ratio, is shown by comparison of the results from diets XXII and XXIII with those from diets XIX, XX and XXI. Diet XXII (normal Ca and P but low Fe) produced an erythrocyte picture similar to that with diets XX and XXI but the terminal hemoglobin level, although low, was higher than that

for the other abnormal diets. Since the hemoglobin values from diet XXIII (low Ca, normal P, and low Fe) were definitely lower than those from diet XXII it is demonstrated that a relative excess of phosphorus decreases hematopoiesis whether the level of dietary iron is generous or sub-minimal for normal blood formation.

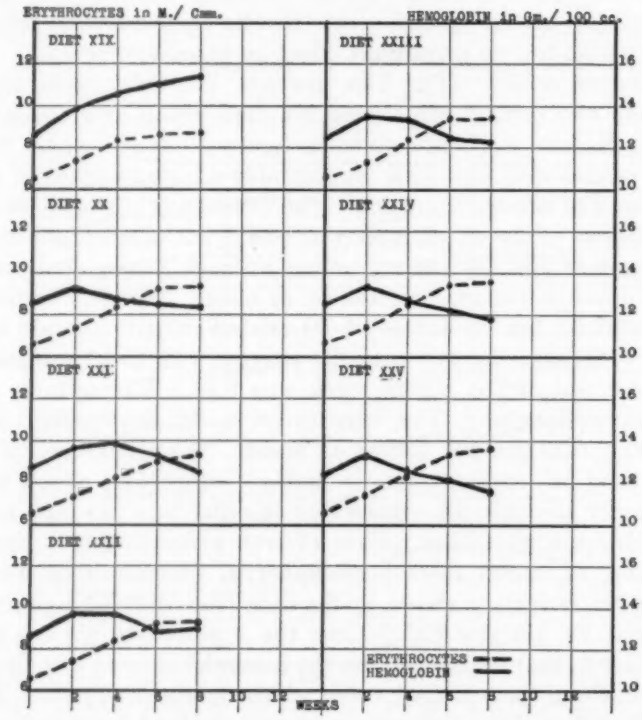

Fig. 2 Average erythrocyte and hemoglobin content of blood from rats maintained on a ration deficient in Ca, P, Fe and vitamin D supplemented as follows: XIX, 19.5 mg. Ca, 19.5 mg. P, 2.3 mg. Fe, and vitamin D; XX, 19.5 mg. Ca, 58.5 mg. P, 2.3 mg. Fe, and vitamin D; XXI, 3.9 mg. Ca, 19.5 mg. P, 2.3 mg. Fe, and vitamin D; XXII, 19.5 mg. Ca, 19.5 mg. P, 0.23 mg. Fe, and vitamin D; XXIII, 3.9 mg. Ca, 19.5 mg. P, 0.23 mg. Fe, and vitamin D; XXIV, same as XXI but without vitamin D; and XXV, same as XXIII but without vitamin D. Ca furnished as calcium carbonate, P as dihydrogen ammonium phosphate, Fe as ferric chloride, and vitamin D as Viosterol.

All of the above diets contained vitamin D. It was interesting therefore to compare hematopoiesis on diets deficient in this nutrient with those containing it. Diet XXIV (low Ca, normal P and Fe, and without vitamin D) produced a significantly greater degree of anemia and polycythemia than diet XXI which contained vitamin D but in other respects was similar to diet XXIV. Moreover diet XXV (low Ca, normal P, low Fe and without vitamin D) also caused a greater degree of anemia and polycythemia than diet XXIII which contained the same amount of Ca, P and Fe, but included vitamin D. This shows that vitamin D facilitates blood formation when the diet contains a relative excess of phosphorus.

Sex differences in series I and II. An analysis of the data showed that no appreciable sex differences in erythrocyte counts or hemoglobin values occurred in either series I or II animals.

Growth in series I and II. Animals on diets I, II, VI, VII and X showed better growth than those on diets III, IV, V, VIII and IX. The average final weights, in grams, of males in the first group (better growth) were: 182, 178, 176, 178 and 228, respectively. Corresponding values for males in the second group (poorer growth) were 151, 154, 144 and 146 respectively. The final weights of females, although uniformly lower, showed approximately the same differences.

In the second series (II) the average final weights, in grams, of males were: 119, 106, 128, 134, 126, 93 and 91 respectively for animals on diets XIX to XXV inclusive. Thus there were no appreciable growth differences between animals on diets XIX, XXI, XXII and XXIII. Poorer growth should be expected on diets XXIV and XXV since no vitamin D was provided. The retarded growth on diet XX would seem to be related to the high level of phosphorus ingestion.

Probably the lower final weight of rats on diet III was caused by the relative high amount of calcium. Since the total daily intake of phosphorus on this diet was about 10 to 12 mg. and the calcium ingestion was slightly over 50 mg., it is evident

that the Ca : P ratio was decidedly unfavorable. The beryllium carbonate in diets IV, VIII and IX may have removed more phosphorus than was necessary to permit normal hematopoiesis, thereby adversely affecting growth. Similarly the ferric chloride in diet V may have bound a greater amount of phosphorus than was needed to allow blood formation, thus retarding growth.

DISCUSSION

The data here reported indubitably demonstrate that in rats on a low-mineral ration containing a relative excess of phosphorus the mild polycythemia and anemia which occur are attributable to the presence of relatively excess phosphorus, and that the effect of calcium in preventing the hematopoietic abnormality resides only in its ability to bind the phosphorus, thus permitting the dietary iron to be used for hematopoiesis instead of being excreted, presumably as a phosphate. This action of calcium has been demonstrated to be neither invariably favorable nor specific; for calcium as the diphosphate had no effect, and with diets containing only minute amounts of calcium, normal blood formation occurred when other cations (Fe^{+++} and Be^{++}) capable of forming insoluble phosphates, were used. Hence it has been shown that neither calcium deficiency nor an extremely low Ca : P ratio are incompatible with normal hematopoiesis, if the diet contains some factor capable of preventing phosphorus from combining with iron, or even if it contains sufficient iron to permit the binding of excess phosphorus.

Swanson, Timson and Frazier ('35), as pointed out by Orten et al., in studies of hematopoiesis in rats on the Smith low-mineral ration, observed more favorable hematopoiesis when edestin was substituted for casein. But they failed to indicate the significance of the phosphorus reduction, or the relation of phosphorus to iron utilization. Shelling and Josephs ('34) recognized that, a priori, a high phosphorus diet should cause the precipitation and excretion of iron as iron phosphate. However, their experimental results, based

on the use of unsatisfactory milk powder diets, failed to reveal an effect of such magnitude as to be regarded significant. Since the data were preliminary in nature it is not possible, as the authors state, to draw any conclusions from them. Kletzien ('38) claims that phosphorus does not interfere with iron assimilation and he makes the surprising statement that "evidence is accumulating which supports the belief that a low Ca:P ratio improves iron assimilation appreciably." The mechanism of this alleged effect is not explained. The problem is made more confusing by the data of Stearns and Stinger ('37) who claim that in iron metabolism studies of infants "no consistent relationship was observed between the iron retention and the intake of . . . calcium or phosphorus." But the experimental procedures in the latter studies were not comparable to those employed in the present investigation.

The data showing the effect of vitamin D deficiency are of interest. Since vitamin D regulates the absorption and excretion of calcium and phosphorus it is to be expected that this nutritient should affect hematopoiesis.

It is of interest that Maughan ('34) reported a reduction in the hemoglobin level of ricketic chicks. Ultraviolet irradiation was accompanied by a prompt rise in hemoglobin but unfortunately the composition of the diet used was not stated. McDonough and Borgen ('37) were unable to demonstrate an appreciable difference in the hemoglobin level of normal and ricketic chicks. Our observations are not necessarily in disagreement with those of McDonough and Borgen since they used a vitamin D-deficient ration in which the CA:P ratio was apparently normal. In our vitamin D-deficient diets the Ca:P ratio was 1:5, which is definitely abnormal. The relation to human nutrition of the data discussed here cannot be stated since there is need of pertinent clinical investigation.

Caution has been urged in the use of large doses of soluble iron salts in hypochromic anemia (Deobald and Elvehjem, '35; Elvehjem, Siemers and Mendenhall, '35; Brock, '37)

since such treatment might cause a phosphorus deficiency. Indeed Brock has reported brief studies in which phosphorus balance determinations were made on patients treated with about 2 gm. daily of different iron compounds, none of which were phosphates. Although there was an augmentation of the phosphorus excretion Brock believes there is no danger of phosphorus deficiency when large doses of iron are being administered, if the phosphorus intake is generous. Further investigation of this problem is needed.

In view of the experimental data reported here it would seem that some consideration should be given to the possibility that mild iron-deficiency anemia might be caused by dietaries containing minimal amounts of iron and a relative excess of phosphorus. Since calcium is the only practicable cation that can be used to prevent the deleterious effect of excess phosphorus on iron utilization, attention should be given to the provision of an adequate Ca: P ratio. Finally, it does not appear improbable that relative excesses of phosphorus might prevent the efficient utilization of manganese (and perhaps certain other trace elements) since the phosphates of manganese are very insoluble (Becker and McCollum, '38; Blumberg, Shelling and Jackson, '38).

SUMMARY

Investigations have been made of the effect on hematopoiesis in rats of variations in the dietary levels of calcium, phosphorus, iron and vitamin D. The basal ration in one series (I) of experiments was markedly deficient in calcium and moderately low in iron, sodium, potassium, magnesium and chloride, while the phosphorus level was considerably in excess as compared with the content of calcium and iron.

The mild polycythemia and anemia which occur with this diet were only partially prevented by supplementation with 1.4 mg. of iron, as ferric chloride, per rat daily. Six milligrams of iron per rat daily produced normal blood values. The same amount of iron as ferric phosphate was without effect. Fifty milligrams of calcium, as calcium carbonate, per

rat daily prevented the abnormal hematological syndrome but the same amount of calcium, furnished as the diphosphate, was completely ineffective.

In the second series (II) a purified ration was used in which calcium, phosphorus, iron and vitamin D could be varied at will. This ration, with normal levels of calcium, iron and vitamin D, but three times the normal amount of phosphorus, caused a mild polycythemia and anemia. The same basal diet supplemented with normal amounts of phosphorus and iron, but only one-fifth the normal quantity of calcium, caused a similar blood picture.

The absence of vitamin D (Viosterol) from low-calcium normal-phosphorus rations, with normal and low levels of iron, caused a greater degree of polycythemia and anemia than similar diets containing vitamin D.

It has been demonstrated therefore that in rats, calcium per se does not have a unique function in hematopoiesis; that a relative or absolute excess of phosphorus causes mild anemia and polycythemia; and that vitamin D facilitates hematopoiesis, presumably through its effect upon calcium and phosphorus metabolism.

The authors wish to acknowledge their appreciation to Dr. E. V. McCollum for his counsel and constant interest in this investigation.

LITERATURE CITED

AMERICAN MEDICAL ASSOCIATION 1937 Editorial. Calcium and iron in blood formation. J. Am. Med. Assoc., vol. 108, p. 479.

BECKER, J. E., AND E. V. MCCOLLUM 1938 The toxicity of $MnCl_2 \cdot 4H_2O$ when fed to rats. Proc. Soc. Exp. Biol. and Med. (in press).

BLUMBERG, H., D. H. SHELLING AND D. A. JACKSON 1938 The production of manganese rickets in rats. J. Nutrition, vol. 16, p. 317.

BROCK, J. F. 1937 The effects of large doses of iron on the absorption of phosphorus. Clin. Science, vol. 3, p. 37.

BROCK, J. F., AND L. K. DIAMOND 1934 Rickets in rats by iron feeding. J. Pediatrics, vol. 4, p. 442.

COX, G. J., M. L. DODDS, H. B. WIGMAN AND F. J. MURPHY 1931 The effects of high doses of aluminum and iron on phosphorus metabolism. J. Biol. Chem., vol. 92, p. xi.

DAVIDSON, L. S. P., H. W. FULLERTON, J. W. HOWIE, J. M. CROLL, J. B. ORR AND W. GODDEN 1933 Observations on nutrition in relation to anaemia. Brit. Med. J., i, p. 685.

DEOBALD, H. J., AND C. A. ELVEHJEM 1935 The effect of feeding high amounts of soluble iron and aluminum salts. Am. J. Physiol., vol. 111, p. 118.

ELVEHJEM, C. A., A. SIEMERS AND D. R. MENDENHALL 1935 Effect of iron and copper therapy on hemoglobin content of the blood of infants. Am. J. Dis. Child., vol. 50, p. 28.

KLETZIEN, S. W. 1938 The influence of calcium and phosphorus on iron assimilation. J. Nutrition, vol. 15 (Suppl.), p. 16.

MAUGHAN, G. H. 1934 Hemoglobin studies. I. In rachitic chicks: Effect of ultraviolet irradiations. Proc. Soc. Exp. Biol. and Med., vol. 32, p. 389.

MCDONOUGH, K. B., AND D. R. BORGEN 1937 A study of the relation of rickets to anemia. J. Lab. and Clin. Med., vol. 22, p. 819.

ORTEN, J. M., A. H. SMITH AND L. B. MENDEL 1936 Relation of calcium and of iron to the erythrocyte and hemoglobin content of the blood of rats consuming a mineral deficient ration. J. Nutrition, vol. 12, p. 373.

SHELLING, D. H., AND H. W. JOSEPHS 1934 Calcium and phosphorus studies. X. The effect of variations of calcium, phosphorus, and of vitamin D in diet on iron retention in rats. Bull. Johns Hopkins Hosp., vol. 55, p. 309.

SHERMAN, H. C. 1907 Iron in food and its functions in nutrition. U. S. Off. Exp. Sta. Bull. no. 185.

SMITH, A. H., AND P. K. SMITH 1934 Inorganic salts in nutrition. X. Electrolyte balance in the serum of rats receiving a diet deficient in inorganic constituents. J. Biol. Chem., vol. 107, p. 681.

STEARNS, G., AND D. STINGER 1937 Iron retention in infancy. J. Nutrition, vol. 13, p. 127.

SWANSON, P. P., G. H. TIMSON AND E. FRAZIER 1935 Some observations on the physiological adjustment of the albino rat to a diet poor in salts when edestin is the source of dietary protein. J. Biol. Chem., vol. 109, p. 729.

VON WENDT, G. 1905 Untersuchungen über den Eiweiss- und Salz-stoffwechsel beim Menschen. Skand. Arch. f. Physiol., Bd. 17, S. 211.

WALTNER, K. 1927 Über die Wirkung grosser Mengen Eisens. I. Über die Wirkung des Eisens auf die Knockenentwicklung. Biochem. Zeitschr., Bd. 188, S. 381.

CURE OF EXPERIMENTAL CANINE BLACKTONGUE WITH OPTIMAL AND MINIMAL DOSES OF NICOTINIC ACID [1]

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THREE FIGURES

(Received for publication July 29, 1938)

In September, 1937 Elvehjem and his collaborators ('37) published their preliminary report that nicotinic acid would cure canine blacktongue. This has been confirmed by Dann ('37), Street and Cowgill ('37), Smith, Margolis and Margolis ('38), Elvehjem, Madden, Strong and Woolley ('38). The data submitted by these investigators was insufficient to establish the optimal and minimal dose of nicotinic acid, and the present investigation was undertaken to clarify this phase of the problem.

METHOD

Experimental canine blacktongue was produced in adult dogs of mixed breed and both sexes, with an average weight of 16 kilograms, by feeding them the modification of Goldberger's

[1] This is the fifteenth in a series of coordinated studies on pellagra in man and associated deficiency diseases of animals from the Departments of Physiology, Pathology, Bacteriology and Medicine of Duke University with the cooperation of Dr. Y. Subbarow of the Department of Biochemistry of Harvard Medical School. Previous publications are listed in the 'Literature Cited' as Smith ('32), Ruffin and Smith ('34), Smith and Sprunt ('35), Dann ('36), Ruffin and Smith ('37), Smith and Ruffin ('37), Smith, Persons and Harvey ('37), Smith, Ruffin and Smith ('37), Dann ('37), Smith ('38), Smith, Margolis and Margolis ('38), Dann and Subbarow ('38), Harvey, Smith, Persons and Burns ('38), Dann ('38).

[2] The expenses of this work were covered in part by grants from the Rockefeller Foundation of New York and the Lederle Biological Laboratories, Pearl River, New York.

diet which has been used in this laboratory by Smith, Persons and Harvey ('37). The method employed for studying the various dosages of nicotinic acid was one standardized by Harvey, Smith, Persons and Burns ('38), and provides for the administration of the test substance over a period of 10 days, then allowing the animal to continue on the basal black-tongue-producing diet until symptoms reappear. This enables one to judge of the animals' immediate response to nicotinic acid, as well as the ability to store the active principle.

Method of administration

A solution of 5 mg. of nicotinic acid per 1 cc. was used for both oral and parenteral administration, water and physiological saline (0.85%) being used respectively as solvent. In some of the experiments reported here, nicotinic acid solutions autoclaved for 6 hours at 15 pounds pressure were used. The results observed did not differ from those previously obtained with 2½-hour and 5-hour autoclaved samples; the autoclaving failed to diminish appreciably the vitamin potency (Smith, Margolis and Margolis, '38).

With the first, second and occasionally the third consecutive attack of blacktongue, the response to nicotinic acid was quite as dramatic as that previously found for liver extract (fig. 1) but in subsequent attacks nicotinic acid failed to produce an adequate gain in weight. The factors involved in this failure to respond to repeated treatments with nicotinic acid are too complicated to be handled in the present paper. Consequently the observations reported here are limited to dogs in their first or second attacks of blacktongue.

Dose

The following dosages of nicotinic acid were studied: 0.1, 0.2, 0.5, 1.0, 1.5 and 10 mg. per kilogram of body weight of the dog, administered daily over a 10-day period.

The comparative effect of oral and intramuscular administration of nicotinic acid has been studied in fifteen dogs with acute blacktongue, who were given 1.5 mg. of nicotinic acid

per kilogram; five dogs were treated orally and ten intramuscularly. At this dosage the results were practically the same (fig. 2). Doses smaller than 1.5 mg. per kilogram were always given intramuscularly.

Five dogs with acute blacktongue, treated intramuscularly with 0.1 mg. of nicotinic acid per kilogram of body weight daily for 10 days, failed to show a return of appetite and continued to lose weight. However, the weight drop was not

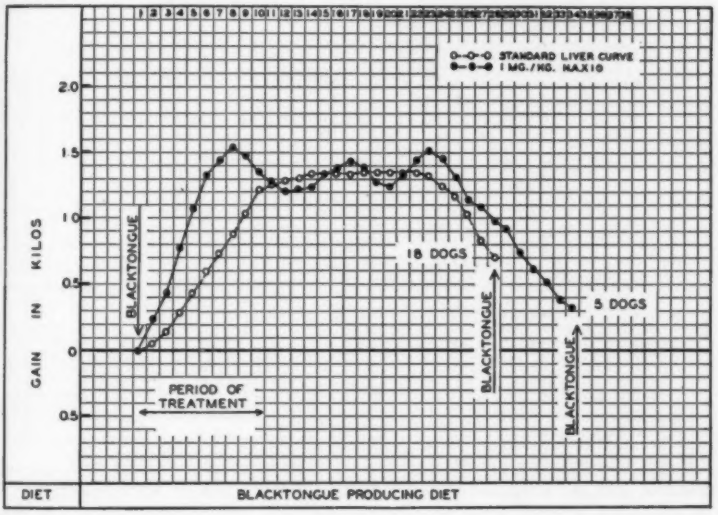

Fig. 1 Comparative growth responses of blacktongue dogs treated with 1) liver extract and 2) nicotinic acid.

so precipitous as that preceding the treatment. The progress of the mouth lesions was arrested and partial healing took place. All of these dogs were subsequently cured with larger doses of nicotinic acid (table 1, fig. 3).

Five dogs with acute blacktongue, receiving intramuscularly 0.2 mg. of nicotinic acid per kilogram of body weight daily for 10 days, ate absolutely no food for 2 days preceding treatment nor for 4 days after treatment was started, yet the

mouth symptoms were completely cured during this time. The appetite remained capricious about 4 days longer, then rapidly returned to normal. The weight remained stationary or decreased for from 5 to 8 days, then increased to an average gain of 0.81 kilogram. A relapse followed this treatment after an average of 19 days.

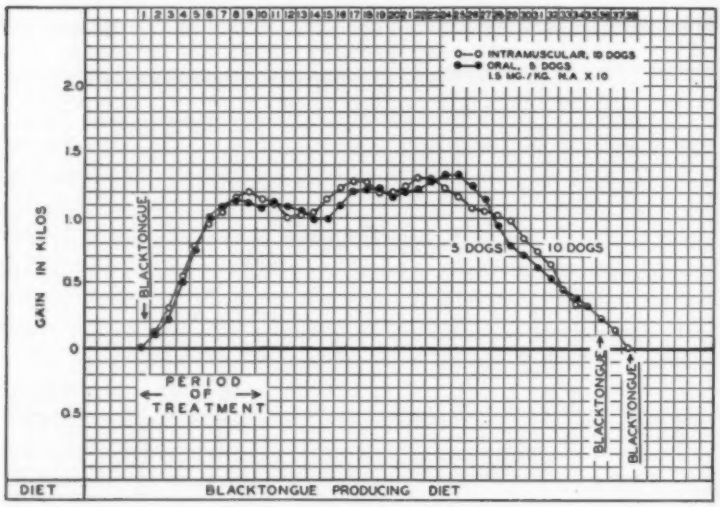

Fig. 2 Comparative growth response of blacktongue dogs treated with 1.5 mg. nicotinic acid per kilogram of body weight 1) orally and 2) intramuscularly.

TABLE 1

Total growth response of blacktongue dogs treated with nicotinic acid

DAILY DOSE, MG./KG. BODY WEIGHT	NUMBER OF DOGS	NUMBER OF TREATMENTS	PERIOD OF TREATMENT	WEIGHT GAIN FROM TREATMENT	TIME BEFORE RELAPSE
			days	*kg.*	*days*
0.1	5	5	10	...[1]	..[2]
0.2	5	5	10	0.81	19
0.5	5	6	10	1.93	25
1.0	3	6	10	1.98	27
1.5	4	8	10	1.64	27
10.0	4	5	10	2.25	29

[1] Continued loss of weight.

[2] Blacktongue not cured.

Five dogs with six consecutive attacks of blacktongue, receiving as treatment for each attack 0.5 mg. of nicotinic acid per kilogram of body weight for a period of 10 days, were cured of their oral lesions in 2 to 4 days. The appetite was restored in 24 to 48 hours, and there was a rapid gain in weight, averaging a total of 1.93 kg. Recurrence of blacktongue symptoms followed this treatment after an average of 25 days.

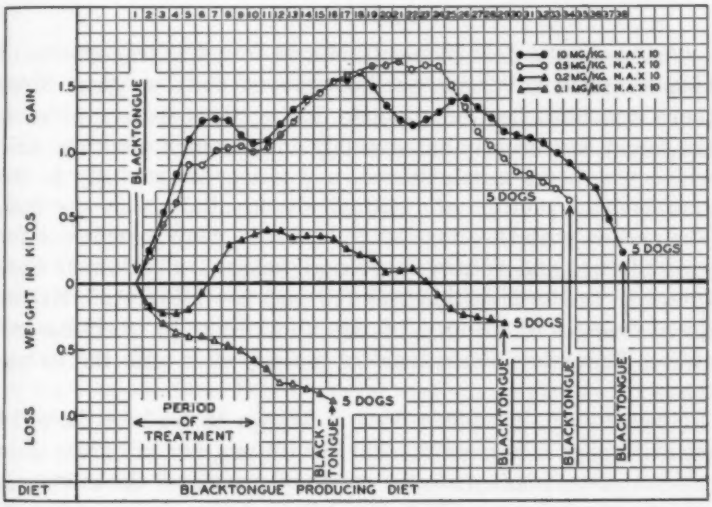

Fig. 3 Comparison of growth response of blacktongue dogs treated with different dose levels of nicotinic acid as follows: 0.1 mg., 0.2 mg., 0.5 mg. and 10.0 mg. per kilogram of body weight, daily for 10 days.

Three dogs, each with two consecutive attacks of acute blacktongue, received as treatment for each attack 1 mg. of nicotinic acid per kilogram of body weight for a period of 10 days, with results similar to those obtained with 0.5 mg. of the material, except that a subsequent relapse in this case was delayed for 27 days, and the average weight gain was 1.98 kg.

Four dogs, each with two consecutive attacks of acute blacktongue, receiving as treatment for each attack 1.5 mg. of nicotinic acid per kilogram of body weight for a period of

10 days, gave responses practically identical with the dogs who received 1 mg. per kilogram.

Four dogs, one of which had two consecutive attacks of acute blacktongue, receiving 10 mg. of nicotinic acid per kilogram of body weight, made practically the same response as did the dogs receiving 0.5 mg., 1.0 mg. and 1.5 mg. All of these responses compare favorably with those obtained with liver extract as observed in a previous experiment (fig. 1).

DISCUSSION

The minimum dose of nicotinic acid found to be curative of blacktongue was 0.2 mg. per kilogram of body weight. Since there was total loss of appetite during the curative process, the effect can be attributed solely to the pure nicotinic acid which was being administered, and not even partially to the supplementary effect of residual vitamin factors in the diet. The results with this dose level, however, were insufficient for prompt cure and return to normal weight. The lowest dose found to accomplish this was 0.5 mg. per kilogram. Subsequent dosages of 1.0 mg., 1.5 mg. and 10 mg. gave similar results. There was no evidence of toxicity even with the 10 mg. dose.

Since the maintenance dose is known to be less than the curative dose it is possible that the 0.1 mg. per kilogram dose which proved inadequate for cure approximates the minimum maintenance dose. Although more work must be done to establish the minimum it is interesting to note that Sebrell ('38) has been able to maintain one dog over a 6 months' experimental period without frank symptoms of blacktongue on a daily dose of 0.12 mg. of nicotinic acid per kilogram of body weight.

Pellagra has been treated successfully with nicotinic acid by Fouts, Helmer, Lepkovsky and Jukes ('37), Smith, Ruffin and Smith ('37), Harris ('37), Spies, Cooper and Blankenhorn ('38), and France, Bates, Barker and Matthews ('38). All of these authors except Smith, Ruffin and Smith, and France, Bates, Barker and Matthews, used doses of from approximately 500 to 1000 mg., or about 7 to 14 mg. per kilo-

gram. The dose used by Smith, Ruffin and Smith ('37), of 60 to 90 mg., or 1.5 mg. per kilogram, was based on the work reported in this paper. France et al. used with good results a similar dose apparently based upon the earlier report of Smith, Ruffin and Smith. This is less than one-fifth the 'experimental dose' recommended recently by the Council on Pharmacy and Chemistry of the American Medical Association (1938).

SUMMARY AND CONCLUSIONS

Twenty-six dogs were studied in thirty-five attacks of acute blacktongue in their response to treatment with nicotinic acid at various dose levels under standardized conditions.

The minimum effective daily dose of nicotinic acid as judged by growth response, storage, prompt return of appetite and disappearance of the classical mouth symptoms was 0.5 mg. per kilogram. Daily doses of 1.0, 1.5 and 10 mg. per kilogram of body weight were equally effective. When the dose was reduced to 0.2 mg. per kilogram daily, the curative response was delayed. The weight remained constant or decreased during the first 5 to 8 days of treatment while the mouth lesions healed, then there was a gradual moderate gain in weight with return of appetite. A dose of 0.1 mg. per kilogram daily was completely ineffective in restoring the appetite and in preventing the loss of weight, but was sufficient to arrest the progress of and partially heal the mouth lesions, at the same time decreasing the rate of the weight loss.

A solution of nicotinic acid autoclaved for 6 hours was just as effective even at the lower levels of 0.2 mg. and 0.5 mg. as was the unautoclaved material.

LITERATURE CITED

AMERICAN MEDICAL ASSOCIATION 1938 Council on Pharmacy and Chemistry. Nicotinic acid. J. Am. Med. Assoc., vol. 111, p. 27.

DANN, W. J. 1936 The vitamin G complex; the non-identity of rat dermatitis due to vitamin B_6 deficiency and the dermatitis of human pellagra. J. Nutrition, vol. 11, p. 451.

———— 1937 Nicotinic acid and vitamin B_6. Science, vol. 86, p. 616.

———— 1938 Animal deficiency diseases related to pellagra. J. Am. Dietetic Assoc., vol. 14, p. 157.

DANN, W. J., AND Y. SUBBAROW 1938 Differentiation of the rat dermatitis factor and the chick dermatitis factor from nicotinic acid. J. Nutrition, vol. 16, p. 183.

ELVEHJEM, C. A., R. J. MADDEN, F. M. STRONG AND D. W. WOOLLEY 1937 Relation of nicotinic acid and nicotinic amide to canine blacktongue. J. Am. Chem. Soc., vol. 59, p. 1767.

———— 1938 The isolation and identification of the anti-blacktongue factor. J. Biol. Chem., vol. 123, p. 137.

FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES 1937 Treatment of human pellagra with nicotinic acid. Proc. Soc. Exp. Biol. and Med., vol. 37, p. 405.

FRANCE, R., R. D. BATES, W. H. BARKER AND E. MATTHEWS 1938 Two cases of pellagra treated with nicotinic acid. Johns Hopkins Hospital Bull., vol. 63, p. 46.

HARRIS, L. J. 1937 Nicotinic acid and the pellagra preventing ('P.P.') vitamin. Chem. Ind., vol. 56, p. 1134.

HARVEY, H. I., D. T. SMITH, E. L. PERSONS AND M. V. BURNS 1938 The effect of various fractions of liver on experimental canine blacktongue. J. Nutrition, vol. 16, p. 153.

RUFFIN, J. M., AND D. T. SMITH 1938 Personal communication.

———— 1934 The treatment of pellagra with certain preparations of liver. Am. J. Med. Sci., vol. 187, p. 512.

———— 1937 A clinical evaluation of the potency of various extracts of liver in the treatment of pellagra. So. Med. J., vol. 30, p. 4.

SEBRELL, W. H., R. H. ONSTOTT, H. F. FRASER AND F. S. DAFT 1938 Nicotinic acid in the prevention of blacktongue of dogs. J. Nutrition, vol. 16, p. 355.

SMITH, D. T., E. L. PERSONS AND H. I. HARVEY 1937 On the identity of the Goldberger and Underhill types of canine blacktongue; secondary fuso-spirochetal infection of each. J. Nutrition, vol. 14, p. 373.

SMITH, D. T., AND J. M. RUFFIN 1937 Effect of sunlight on the clinical manifestations of pellagra. Arch. Int. Med., vol. 59, p. 631.

SMITH, D. T., J. M. RUFFIN AND S. G. SMITH 1937 Pellagra successfully treated with nicotinic acid. J. Am. Med. Assoc., vol. 109, p. 2054.

SMITH, S. G. 1932 A new symptom complex in vitamin G deficiency in rats. Proc. Soc. Exp. Biol. and Med., vol. 30, p. 198.

———— 1938 Etiology of sebaceous gland atrophy in the rat in avitaminosis. J. Nutrition, vol. 15, p. 45.

SMITH, S. G., G. MARGOLIS AND L. H. MARGOLIS 1938 The effect of autoclaving on vitamin potency of nicotinic acid. Proc. Soc. Exp. Biol. and Med., vol. 38, p. 251.

SMITH, S. G., AND D. H. SPRUNT 1935 Pathological skin changes in the tail of the albino rat on a diet deficient in vitamin G. J. Nutrition, vol. 10, p. 481.

SPIES, T. D., C. COOPER AND M. A. BLANKENHORN 1938 The use of nicotinic acid in the treatment of pellagra. J. Am. Med. Assoc., vol. 110, p. 622.

STREET, H. R., AND G. R. COWGILL 1937 The cure of canine blacktongue with nicotinic acid. Proc. Soc. Exp. Biol. and Med., vol. 37, p. 547.

THEORETICAL AND ACTUAL CALORIC REQUIREMENTS [1]

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ONE FIGURE

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The smallest amount of food compatible with health and the occupation of the individual is not known. The importance of such knowledge is suggested from the many people who, for different reasons in health and disease, must reduce their intake of food. Low-wage incomes and obesity are examples in health; diabetes is an example of disease. That the present day standards of food requirements are based upon habits rather than actual caloric needs is suggested from the work of Chittenden ('05) and from comparison of mortality experiences of life assurance companies with best weights and the body-weight standards in general use. That health may be maintained with much smaller amounts of food than are indicated according to the standards in use is shown in the results to be reported here.

METHOD OF INVESTIGATION

Ideally, to determine the smallest amount of food compatible with health and the occupation of the individual, the subject should be kept in a respiration calorimeter for a long period of time. The activities should be in accord with the ordinary occupation and habits of the individual and the intake of food should be carefully measured. This is not

[1] Part of an address delivered at the annual meeting of the American Association for the Advancement of Science, Ottawa, Canada, June 30, 1938.

practical. The next best procedure is to observe a large group of healthy people on carefully prescribed diets. This also is not practical, because of the necessary duration of the experiment and the difficulty of disciplining healthy people with respect to intake of food. An approach to the ideal, however, is found in experiences with diabetics. Because of the disease, these people MUST live upon diets appreciably below their usual habits; unless they do so, they shorten their lives and, for some time before death, require large amounts of insulin. Whether a group of diabetics selected at random is or is not following the diet prescribed carefully is, therefore, reflected in a) the ratio of actual to expected deaths b) the percentage of individuals who require insulin and c) the average dose of insulin. The data to be presented here, therefore, are based upon a study of 500 diabetics in the Clinic for Diabetes at the Montreal General Hospital observed during an average period of 3.5 years.

THEORETICAL CALORIC REQUIREMENTS

In table 1 are recorded the average height and weight of these 500 diabetics from which was calculated the average body surface according to the DuBois formula. The average expenditure of energy under basal metabolism conditions was calculated from the average age according to the DuBois standards. In estimating the average caloric requirements for 24 hours independent of work, it was assumed that the heat production of the body was 10% less during sleep than when awake and that the specific dynamic action of food was approximately 7%. The average energy expenditure of these 500 individuals, independent of work, was thus found to have been 1816 calories per 24 hours.[2]

[2] This, it will be noted, is a large number of calories for basal metabolism and specific dynamic action of food alone. These people, however, were diabetics among whom obesity is very common. They, therefore, have a large body surface. That this is a common experience is shown from the data of another clinic (Joslin, Dublin and Marks, '36; Joslin, '28) from which it may be calculated that the average weight of the 4680 diabetics investigated was 21.3% in excess of the standard. Their average height was 63.1 inches. According to their age, therefore, their average weight was, approximately, 175 pounds.

TABLE 1

Average theoretical caloric requirements of 500 diabetics (at rest but taking food)

Average height (inches)	65.38
Average weight (pounds)	181.24
Average body surface (sq.m.)	1.90
Average age (years)	48.7
Average basal calories (sq./m./hr.)	38.5

Total caloric requirements

Awake (16 hours)	1170.4
Sleep (8 hours)[1]	526.7
Total	1697.1
SDA of food[2]	118.9
Total	1816.0

[1] Ten per cent less per hour than when awake.

[2] Specific dynamic action of food—approximately 7% of total basal heat production.

Assuming that, approximately, 1000 calories per day are expended during occupations involving light work and 1350 calories during moderate work (Sherman, '37; Best and Taylor, '37; Rose, '38; Becker and Hamalainen, '14; Carpenter, '11, and Benedict and Cathcart, '13) the former types of occupation resulted in a deficit of 862 calories, and the latter in 1212 calories, per day. Thus:

	Light work	*Moderate work*
Energy expenditure independent of work	1816	1816
Energy expenditure due to work	1000	1350
Total	2816	3166
Caloric value of diet	1954	1954
Deficit	862	1212

The average duration of these observations was 3.5 years and during this period the average loss of weight was 28.78 pounds. Thus:

	pounds
Average body weight at beginning of treatment	181.24
Average body weight 3.5 years later	152.46
Reduction of weight	28.78

THEORETICALLY EXPECTED LOSS OF WEIGHT

Theoretically, even assuming a maximum energy value of fat (9.3 calories per gram) and that the losses of weight were due entirely to fat (which is unlikely) they should have been very much greater. In fact, the theoretically expected losses were greater than were physically possible. Thus:

Expected loss of weight with light work:

$$\frac{862 \times 365 \times 3.5}{9.3 \times 452} = 261.9 \text{ pounds}$$

Expected loss of weight with moderate work:

$$\frac{1212 \times 365 \times 3.5}{9.3 \times 452} = 368.3 \text{ pounds}$$

The discrepancy between the actual and theoretically expected loss of weight is still more marked, when the actual basal metabolism data are used instead of the calculations shown in table 1. Thus:

The average basal metabolic rate of these 500 subjects was +4.2%. The average heat production, independent of work was therefore, approximately, 1892 calories ($1697.1 \times 1.042 \times 1.07$). The expected losses of weight were, therefore, as follows:

	Light work	*Moderate work*
Energy expenditure independent of work	1892	1892
Energy expenditure due to work	1000	1350
Total	2892	3242
Caloric value of food	1954	1954
Deficit	938	1288

Expected loss of weight with light work:

$$\frac{938 \times 365 \times 3.5}{9.3 \times 452} = 285.1.$$

Expected loss of weight with moderate work:

$$\frac{1288 \times 365 \times 3.5}{9.3 \times 452} = 391.4.$$

It is obvious, therefore, that it is necessary to investigate the generally accepted methods of determining food requirements.

VARIABLES

Muscular activity. A possibility which must be considered is that the allowances of 1000 calories for light work and 1350 calories for moderate work were too great. That they were not too great is suggested from data obtained experimentally under different conditions of muscular activity. Such data show (Rose, '38) that lying absolutely still, but taking food, alone requires about 0.1 calorie per kilogram of body weight, or over 100 calories for 16 hours awake, by a person weighing about 150 pounds. Sitting absolutely still for the same number of hours requires twice this number of calories and sitting at ease for the same number of hours results in an expenditure of over 400 calories. Walking very slowly—at the rate of 1 mile per hour—would alone, in 8 hours, result in an expenditure of over 500 calories. The above estimates of 1000 and 1350 calories for 8 hours of light and moderate work respectively, therefore, appear reasonable. This discrepancy between the theoretically expected and actual losses of weight due to muscular activity alone will be referred to again.

Oedema. Another possibility which must be considered is failure to lose weight because of retention of water—oedema. That retention of water commonly occurs with low-caloric diets is a well-recognized fact. Results of treatment of obesity are, perhaps, the best examples (Newburgh, '31; Wiley and Newburgh, '31). Admittedly, a person may retain 5 to 10 pounds of water without any evidence of oedema (pitting of the skin on pressure, puffiness of the eyelids, etc.). A person cannot, however, retain 25 or more pounds of water without manifesting one or another of these signs. Retention of water is, therefore, excluded as an important factor.

Diet. An important variable which must be considered is the care with which these individuals followed the diets prescribed. As stated previously, unless diabetics follow their diets reasonably carefully, they shorten their lives and, for some time before death, require large amounts of insulin.

Therefore, whether these individuals did or did not follow their diets as prescribed should be reflected in a) their ratio of actual to expected deaths, b) the percentage of individuals who required insulin at the beginning and at the end of the period of observation and c) the average number of units of insulin at the beginning and at the end of the period of observation. The results of this analysis of mortality and insulin dosage are briefly summarized in table 2.

Mortality. It will be noted that of the 500 diabetics, 478 were alive at the end of the 3.5 years. Since their average age at the end of this period of observation was 52.2 years

TABLE 2

Showing relationship between degree of control of diabetes and insulin requirements

GROUP		PRIVATE		PUBLIC	
		At first	3.5 years later	At first	3.5 years later
Whole	Number	500	478	145	145
Insulin	Number	142	128	71	88
	Per cent	28.4	26.8	48.9	60.7
	Average dose (units)	26.4	20.8	30.9	35.6
Diet (calories)		1954		1503	

(table 1) their ratio of actual to expected deaths was practically normal; that is, no more of these 500 diabetics died during this period of 3.5 years than were expected to die according to the 'normal population death rate.'[3] This alone suggests that the diabetes was under good control and, therefore that these people followed their diets reasonably carefully. More significant, however, are the experiences with insulin.

Insulin dosage. Among the deaths there was a number of individuals who had required insulin. This lowered the percentage of individuals who required insulin and would thus mask the effects of dietary irregularities among those alive.

[3] Of every 1000 people alive at age 52 years, approximately 14 are expected to die before their next birthday (U. S. Life Tables, '34).

In fact, the data showed that a number who did not require insulin at the beginning treatment required it later. The data, however, also clearly show that practically an equal number were able to reduce their dosages and some were able to discontinue its use entirely. The significant facts, as the table shows, are a) the percentage of individuals who required insulin was no greater at the end of the 3.5 years than at the beginning of treatment and that b) the average dose was definitely reduced. Reduction of insulin dosage definitely indicates improvement of carbohydrate tolerance and is opposed to an intake of food appreciably greater than prescribed.

Opposed also to gross dietary irregularities are experiences with the control (public) group of diabetics who were purposely selected because they were unable to follow treatment as carefully as prescribed. The fact that the average number of calories ALLOWED in this control group was smaller than in the 500 cases—1503 compared with 1954—alone suggests the difficulties met with in the attempts to counterbalance the irregularities. It will be noted, however, that in spite of the smaller number of calories among those who for different reasons did not follow treatment as carefully as prescribed, not only was the percentage of individuals who required insulin greater both at the beginning and the end of the 3.5 years of observation, but the average number of units was also greater.[4] The conclusion, therefore, is that consumption of more food than prescribed does not account for the discrepancy between the theoretically expected and actual losses of weight.

[4] The fact that the control group required more insulin at the beginning of the period suggests that they were more severe diabetics. Actually, the larger dosage is due to the method used to collect the data in these cases. In the selection of the 500 cases the beginning of the period of observation corresponded with the beginning of treatment with insulin. In the selection of the control group, the most recent insulin dosage was compared with that of 3.5 years before. The important fact in the present investigation is that whereas, the average dose of insulin was lower at the end than at the beginning of the 3.5 years among the 500 cases, it was greater in the control group, in spite of the smaller number of calories.

Reduction of basal metabolism. Benedict and his associates ('19) demonstrated that underfeeding lowers the basal metabolism. A summary of the data (Lusk, '28) shows that after 3 weeks only with a diet of 1375 calories the energy expended under basal metabolism conditions was reduced from 1745 to 1293 calories—approximately 32%. A still more marked reduction of basal metabolism following undernutrition was reported by Gordon and Rabinowitch ('30). The possible effects of reduction of basal metabolism in these

TABLE 3

Average reduction of basal metabolism of 500 diabetics following treatment with low calorie diets

	BASAL METABOLIC RATE (PER CENT OF NORMAL)	
	Before treatment	After treatment [1]
Maximum	122.3	116.2
Minimum	81.6	84.0
Average	104.2	92.3
Standard deviation	26.3	21.8
Probable error of mean	0.79	0.65
Difference between means		11.9
Probable error of difference		1.02
$\frac{\text{Difference between means}}{\text{Probable error of difference}}$		11.6

[1] Three and one-half years.

500 diabetics due to the low-calorie diets were, therefore, investigated and the results are briefly summarized in table 3. It will be noted that the average basal metabolic rate was reduced from +4.2 to −7.7%. That this difference of 11.9% was significant is shown by the ratio of the difference to its probable error.

The above difference of 11.9% does not indicate the total conservation of body weight due to the reduction of basal metabolism. It is important to note that the average weight of these subjects was 181.24 pounds at the beginning of the period of observation, whereas, 3.5 years later, it was 152.46

pounds. Since the basal metabolic rate is calculated from the body surface and since the latter is influenced to a large extent by body weight, it is necessary to recalculate the energy expenditure per hour according to the new body surface due to the reduction of weight. It is also necessary to correct for the lower caloric requirements due to older age. The combinded data are shown in table 4. It will be noted that, because of the reduction of weight, the requirements per 24 hours,

TABLE 4

Showing reduction of caloric requirements due to reduction of basal metabolism

	BEFORE TREATMENT	3.5 YEARS LATER
Basal metabolism (per cent)	+4.2	−7.7
Age (years)	48.7	52.2
Standard calories per sq.m./hr.	38.5	37.5
Height (inches)	65.4	65.4
Weight (pounds)	181.2	152.5
Body surface (sq.m.)	1.90	1.77
Standard calories per hour	73.1	66.4
Actual calories per hour	76.2	61.3
8 hours sleep[1]	548.6	441.4
16 hours awake	1219.2	980.8
Total	1767.8	1422.2
Specific dynamic action of food[2]	123.7	99.5
Total	1891.5	1521.7

[1] Heat production per hour during sleep 10% less than when awake.
[2] Approximately 7% of total basal heat production.

independent of work, were reduced from 1892 to 1522 calories —approximately, 19.5%. This alone, if it operated for a period of 3.5 years, accounts for a conservation of, approximately, 112 pounds of body weight. Thus:

$$\frac{(1891.5 - 1521.7) \times (365 \times 3.5)}{452 \times 9.3} = 112.5$$

It is, therefore, necessary to take into consideration the fact that the basal metabolism and body weight may be reduced very rapidly—within a few weeks (Lusk, '28)—by low-calorie diets. Assuming, however, that the basal metabolism was reduced to its LOWEST level (−11.9% or 1522

calories per 24 hours) within a few weeks and that ALL of the loss of body weight occurred very early in treatment, there is still a marked discrepancy between the actual and theoretically expected loss of weight. Thus:

	Light work	*Moderate work*
Caloric requirements independent of work (table 4)	1522	1522
Work (calories)	1000	1350
Total	2522	2872
Caloric value of food	1954	1954
Deficit	568	918
Theoretically expected loss of weight (pounds)	172.7 [5]	279.1 [6]

Specific dynamic action of food. All of these subjects were diabetics and the majority were obese when first observed. The specific dynamic action of food is alleged to be reduced in obesity [7] and also in pituitary disease—conditions closely related to diabetes. Consideration must, therefore, be given to possible conservation of weight due to impaired specific dynamic action.

A saving of one calorie a day for a period of 3.5 years could account for a conservation of 0.304 pounds. Thus:

$$\frac{1 \times 365 \times 3.5}{452 \times 9.3} = 0.304.$$

In table 4 it was noted that specific dynamic action of food accounted for an expenditure of, approximately, 100 calories per day. Therefore, assuming even COMPLETE ABSENCE of specific dynamic action in all of these 500 diabetics during the ENTIRE period of 3.5 years, the total conservation of body weight would have been about 30 pounds only. Allowing for the actual loss of weight of 29 pounds and 10 pounds for retention of water without oedema, it is, therefore, still necessary to account for about 100 pounds with light work and over 200 pounds with moderate work.

Protein metabolism. Failure to maintain nitrogen equilibrium is a cause of weakness, fatigue and ill-health in

[5] $\frac{568 \times 365 \times 3.5}{4.52 \times 9.3}$

[6] $\frac{918 \times 365 \times 3.5}{4.52 \times 9.3}$

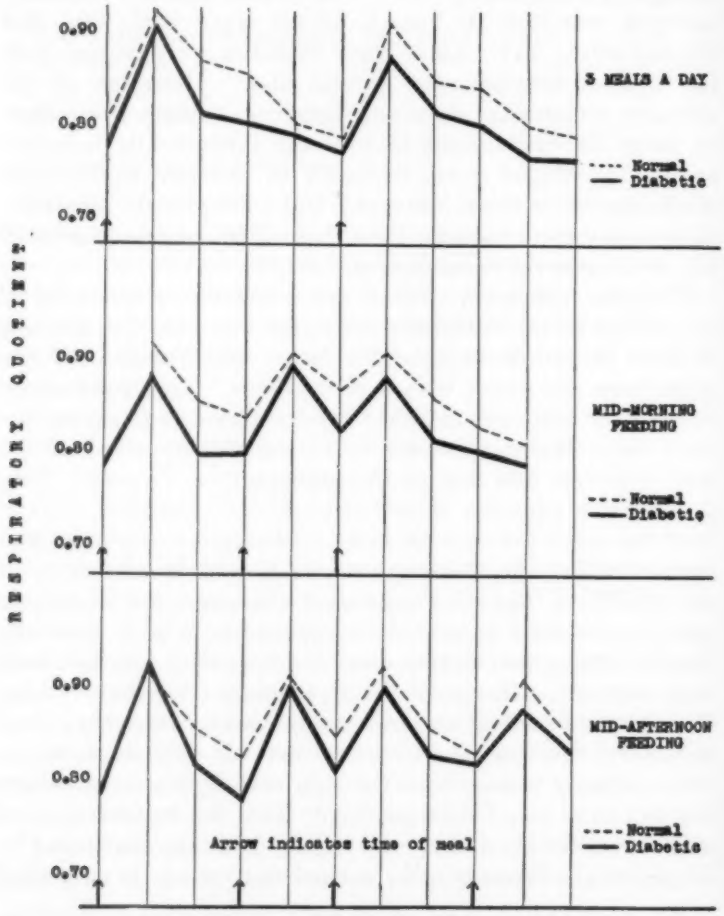
[7] We have been unable to confirm this observation in this laboratory.

general in diabetics (Rabinowitch, '36). It is also a well-known fact that it is difficult to maintain nitrogen equilibrium with high fat diets; whereas, one of the most striking effects of the high carbohydrate-low calorie diet is retention of nitrogen (Rabinowitch, '30) The lowest values of urinary nitrogen reported in human beings were with this diet (Rabinowitch, '31). All of these diabetics were treated with the high carbohydrate-low calorie diet. Alteration of the nitrogen metabolism does not, however, explain the failure of these 500 individuals to lose the theoretically expected amount of weight since, by being in nitrogen equilibrium, conditions were merely normal, and normal individuals require many more calories than these diets contained according to the generally accepted standards.

Muscular efficiency. There are a number of methods of conserving fuel in the human body (Rabinowitch, '38) and one of these is the character of the food. The human body has often been compared with a coal engine. This comparison has a very little foundation. To act like a coal engine the body must operate between two temperatures—that of the heat reservoir and that of the condensor or the sink. This, as Carnot's equation shows (Burns, '21; Feldman, '23) it does not do. The human body, however, resembles a heat engine in that its efficiency may be altered by alteration of the TYPE of fuel; for maximum efficiency, carbohydrates must be the chief source of energy—work is done most efficiently with carbohydrates, less efficiently with fats and least with proteins. Haggard and Greenberg ('35) have shown that frequent feeding also increases muscular efficiency. It is necessary, therefore, here to note that all of these diabetics were not only treated with the high carbohydrate-low calorie diet but were also fed frequently.[8] That the improvement of efficiency with this diet is only slightly less than that noted by Haggard and Greenberg in normal individuals is suggested

[8] Though there have been no radical changes of this diet since it was first reported (Rabinowitch, '30), it has been made more attractive by an increase of protein and frequent feedings. Its general effects upon health and improvement of carbohydrate tolerance have been reported elsewhere (Rabinowitch, '32).

from the parallelism of the respiratory quotients. These are shown in the accompanying figure. The maximum muscular efficiency attainable in man, however, appears to be about 28 to 30% only (Haggard and Greenberg, '35; Rubner, '28), and



Normal subjects: Haggard and Greenberg, '35.

Fig. 1 Showing parallelism between hourly respiratory quotients obtained during frequent feeding of normal individuals and during similar feeding of diabetics under control.

by frequent feeding of carbohydrate it appears that the maximum conservation of fuel is about 7% only (Haggard and Greenberg, '35). With light work, the frequent feeding could, therefore, have effected a saving of about 70 calories only and with moderate work about 100 calories only per day and thus have resulted in a conservation of about 20 pounds only with light work and 30 pounds only with moderate work. There is, therefore, still a very appreciable amount of weight to account for—over 80 pounds with light work and about 180 pounds with moderate work.

Calories and work. The law of the conservation of energy applies to the human body as elsewhere. Experimentally, this was demonstrated by Atwater and Benedict ('03). The few calories which were available for work in these 500 cases may, therefore, be seen in the following formula which expresses the law of the conservation of energy in man:

$$Lf + F = B + dB + M + M\left(\frac{1-e}{e}\right)$$

where

L = loss of weight (grams)
f = 9.3 (caloric value of 1 gm. fat)
F = caloric value of diet
B = energy expenditure under basal conditions
d = specific dynamic action of diet
M = mechanical work
e = efficiency of human body

It will be noted (table 5) that assuming an efficiency of 25%, 58.1 calories only appear to have been available for work at the beginning of treatment and 108.1 calories only 3.5 years later. Even assuming immediate reduction of the basal metabolism to its lowest level, owing to the underfeeding, approximately, 131.7 calories only were available for work.

Theoretically, very few calories should be required for mechanical work. According to the mechanical equivalent of heat, 427 kilogram-meters of work are equal to one calorie. One calorie is, therefore, sufficient energy to raise a 1-pound weight about 3050 feet. One-half teaspoonful of sugar (10 calories) therefore, contains sufficient energy to raise a 1-pound weight to the top of Mt. Everest—approximately 30,000 feet. A diet which contains about 100 calories in

excess of the requirements for the basal metabolism and of the expenditure of energy due to the specific dynamic action of the food is, therefore, sufficient to perform about 300,000 foot-pounds of work.

The above caluculations, however, do not take into consideration the mechanical efficiency of the human body. In the calculations shown in table 5 it was assumed that the latter is approximately 25%. This, it should be noted, is the average NET efficiency of MUSCLE; it is not the GROSS efficiency;[9] nor does it reflect the efficiency of the body as a whole. That the latter is much lower than 25% is suggested from the average intake of food; in a diet of 2500 calories

TABLE 5

Total energy available per day for mechanical work according to the equation which expresses the law of the conservation of energy in the human body

TIME	TOTAL AVAILABLE ENERGY		EXPENDITURE OF ENERGY INDEPENDENT OF WORK		ENERGY AVAILABLE FOR WORK		
					Total	Available	Wasted
	Lf	F	B	dB	$M+M\frac{1-e}{e}$	M	$M\frac{1-e}{e}$
Before tdeatment	94.4	1954	1697.1	118.9	232.4	58.1	174.3
Soon after [1]	94.4	1954	1422.2	99.5	526.7	131.7	395.0
3.5 years later	0	1954	1422.2	99.5	432.3	108.1	324.2

[1] Assuming rapid reduction of basal metabolism to lowest level.

per day, there are available for mechanical work approximately 1000 calories. This amount is equivalent to about 3,000,000 foot-pounds.

Assuming that the efficiency of the human body as a whole is much lower than 25%, it is necessary to find a source of energy other than the food supply to account for the failure of these 500 diabetics to lose the theoretically expected weight. One possible source suggested is some of the potential energy of muscular tension. That the body can conserve energy which is ordinarily dissipated as heat and convert it into useful work is suggested from the reduction and, at times, complete absence of the specific dynamic action of carbohydrate food during work (Rose, '38; Lusk, '28).

[9] Gross efficiency is not measurable because no external work is done during the period of 'inactivity.'

Whether the efficiency of the human body as a whole is greater or less than the known efficiency of muscle must be determined experimentally and no experiment has as yet been devised for this purpose. The importance of this experiment is shown by the experiences with the above-mentioned 500 diabetics. These experiences indicate that bodily equilibrium can be maintained with diets the caloric values of which are much below the generally accepted standards.

SUMMARY

From the experiences with 500 diabetics, it is shown that bodily equilibrium may be maintained with diets the caloric contents of which are much below the generally accepted standards. With these diets, the average loss of weight was very much less than expected theoretically.

Conditions which might have accounted for the discrepancy between the actual and theoretically expected loss of weight are briefly discussed. It is shown that even by assuming a) reduction of the basal metabolism to its lowest level within a few weeks, b) complete absence of specific dynamic action of food in all cases during the entire period of obsevation, c) maximum muscular efficiency throughout this period and d) that the occupations of all of these diabetics consisted of light work only, there was still an appreciable difference between the actual and theoretically expected loss of weight.

According to the equation which expresses the law of conservation of energy in man, it is shown that the amount of energy available for work in the diets of these individuals was very small. Since these diets resulted in improvement, and not in impairment, of health, it is necessary to assume that the human body is very efficient—that when necessary very little of the potential energy of the food is wasted—or that with a tendency toward underfeeding the body has available some unrecognized source of energy. Since the average diet contains sufficient energy (about 2500 calories) to do about 3,000,000 foot-pounds of work, independent of the basal metabolism requirements the human machine appears to be very inefficient under ordinary conditions. The alternative, therefore, appears to be that with a tendency toward

underfeeding, the body has available some unrecognized source of energy. That the body can, at times, use energy which is ordinarily wasted as heat is suggested from the reduction of the specific dynamic action of food during work.

LITERATURE CITED

ATWATER, W. O., AND F. G. BENEDICT 1903 Experiments on the metabolism of matter and energy in the human body. U. S. Dept. Agric., Bull. no 136, p. 1.

BECKER, G., AND HAMALAINEN 1914 Science of nutrition. 4th ed., p. 463.

BENEDICT, F. G., W. R. MILES, P. ROTH AND H. M. SMITH 1919 Human vitality and efficiency under prolonged restricted diet. Carnegie Inst. Wash., pub. no. 280, p. 1.

BENEDICT, F. G., AND E. P. CATHCART 1913 Muscular work. Carnegie Inst. Wash., pub. no. 187, p. 1.

BEST, C. H., AND N. B. TAYLOR 1937 Physiological basis of medical practice. P. 862.

BURNS, D. 1921 An introduction to biophysics, p. 33.

CARPENTER, T. M. 1911 The increase in metabolism due to the work of typewriting. J. Biol. Chem., vol. 9, p. 231.

CHITTENDEN, R. H. 1905 Physiological economy in nutrition.

FELDMAN, W. M. 1923 Biomathematics, p. 235.

GORDON, A. H., AND I. M. RABINOWITCH 1930 Low basal metabolism following lobar pneumonia with marked undernutrition. Am. J. Med. Sci., vol. 180, p. 695.

HAGGARD, H. W., AND L. A. GREENBERG 1935 Diet and physical efficiency.

JOSLIN, E. P. 1928 The treatment of diabetes mellitus. 4th ed., p. 157.

JOSLIN, E. P., L. I. DUBLIN AND H. H. MARKS 1936 Studies in diabetes mellitus. Am. J. Med. Sci., vol 192, p. 9.

LUSK, G. 1928 The science of nutrition. 4th ed., p. 173.

NEWBURGH, L. H. 1931 The cause of obesity. J. Am. Med. Assoc., vol. 97, p. 1659.

RABINOWITCH, I. M. 1930 Experiences with a high carbohydrate-low calorie diet for the treatment of diabetes mellitus. Can. Med. Assoc. J., vol. 23, p. 489.

———— 1931 Clinical and laboratory experiences with a high carbohydrate-low calorie diet in the treatment of diabetes mellitus. New Eng. J. Med., vol. 204, p. 799.

———— 1932 The present status of the high carbohydrate low calorie diet for the treatment of diabetes. Can. Med. Assoc. J., vol. 26, p. 141.

———— 1936 Medical complications of diabetes mellitus. J. Med. Soc., New Jersey, September number.

———— 1938 Calories and occupation. Can. Med. Assoc. J., vol. 38, p. 277.

ROSE, M. S. 1938 The foundations of nutrition. 3rd ed., p. 49.

RUBNER 1928 The science of nutrition. 4th ed., p. 409.

SHERMAN, H. C. 1937 Chemistry of food and nutrition. 5th ed., p. 206.

WILEY, F., AND L. H. NEWBURGH 1931 An improved method for the determination of water balance. J. Clin. Invest., vol. 10, p. 723.

VARIATION OF WEIGHT OF DRY FECES IN SHORT PERIOD EXPERIMENTS WITH A LOW RESIDUE-NEUTRAL ASH DIET [1]

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A variety of food experiments and investigations of nutrition in general include measurement of feces and a number of conditions which increase or decrease its weight—cellulose, hemicellulose and lignin contents of diet, etc.—are now well known. The average weight of dried feces of normal individuals on ordinary diets is not very large but fluctuates widely, ranging between 25 and 60 gm. per day. Given a diet of constant composition, the probability is that, if continued for a long time (weeks) the daily weight of the dried feces is quite constant or fluctuates within a very narrow range. Judging from the literature, however, there appear to be no data with respect to variations of weight of dried feces with diets of constant composition but of short duration (days). The importance of such information is obvious from the fact that the majority of experiments which involve fecal analyses are of short duration.

In a previous paper in this Journal (Rabinowitch, '32) one of the writers (I.M.R.) reported the mineral metabolism in a case of osteitis deformans. The data included analyses of intake and output of sodium, potassium calcium, magnesium, chlorine, phosphorus and sulphur. In order to de-

[1] This work was done with the aid of a grant from Mr. J. C. Newman, President, and Mr. Julian C. Smith, member of the Board of Management, of The Montreal General Hospital.

termine the influence of the disease upon the excretion of these elements, it was necessary to control the residue of the diet, since, as is well known, large residues tend to increase peristalsis and result in loss of materials in the feces. The reaction of the ash also had to be controlled since, acid-ash diets tend to increase, whereas alkaline-ash diets tend to decrease, excretion of calcium, etc. The daily diet in the above experiment consisted of the following:

	gm.
Bread	420
Butter	85
Eggs	200
Milk	1680
Orange juice	200

The low residue content is obvious. That this diet also yielded a practically neutral ash may be seen from the following table:

TYPE	AMOUNT (GM.)	EXCESS ACID (CC. N/10)		EXCESS BASE (CC. N/10)	
		Per 100 gm.	Total	Per 100 gm.	Total
Eggs	200	11.1	22.2	...	....
Milk	1680	....	...	2.37	39.80
Bread (white)	420	7.36	30.9	...	....
Oranges	200	....	...	5.61	11.22
Total (cc. N/10)			53.1		51.0

After the work of the above-mentioned experiment had been completed, it was accidentally observed that though the amounts of the wet feces differed widely, the average weights of the dried materials per day were remarkably alike in the two periods of the experiment, in spite of their short duration. Thus:

PERIOD	WEIGHT OF DRIED FECES (GM.)	
	Total	Per day
February 6 to 11	118.2	19.7
March 9 to 14	114.4	19.0

Because of these findings it was considered of interest to investigate the data of a somewhat similar study in a case of

hyperparathyroidism, the findings of one period of which are briefly summarized in the above-mentioned paper (Rabinowitch, '32). Here, also, though the amounts of the wet feces differed widely in the two periods of the experiment, the weights of the dried materials were remarkably alike. Thus:

PERIOD	WEIGHT OF DRIED FECES (GM.)	
	Total	Per day
First period	223.0	22.3
Second period	219.7	21.9

Since the above observations, a variety of mineral balance experiments have been performed. In this report we are, however, concerned only with the weights of the dried feces.

METHOD OF INVESTIGATION

The details of management were the same in each case. All of the necessary precautions with respect to the diet, nursing care in general and quantitative collection of feces otherwise were adhered to strictly as previously described (Rabinowitch, '32). In each case it was also made certain that every meal was completed; there were no residues.[2]

In all, the data reported here include twenty experiments in eleven different subjects with eight different clinical conditions. The periods of the experiments ranged between 3 and 20 days. The diet, however, was the same in each case and as described above. The number of days of each experiment, the total weight of the dried feces and the average weight per day are shown in table 1.

RESULTS

The results require little comment. It will be noted that with the above-mentioned low residue-neutral ash diet, in spite of the short periods of the experiments, the average weight of dry feces per day was remarkably the same not only

[2] The menu with the food materials allowed on this diet must be made attractive in balance experiments in order to avoid residues and thus numerous additional analyses. A variety of dishes is possible—egg sandwiches, eggnogs, custards, etc.

TABLE 1

Showing constancy of weight of dry feces

EXPERIMENT NO.	HOSPITAL NO.	CLINICAL DIAGNOSIS	PERIOD	NUMBER OF DAYS	WEIGHT OF DRY FECES	
					Total	Per day
					gm.	*gm.*
1	x	Hyperparathyroidism	1	10	223.5	22.3
2			2	10	219.7	21.9
3	454/31	Osteitis deformans	1	6	137.5	22.9
4	454/31		2	6	143.4	23.9
5	2424/32	Anaemia	1	7	136.0	19.4
6	2424/32		2	7	135.0	19.3
7	4052/32	Anaemia	1	7	160.0	22.8
8	4052/32		2	7	142.0	20.3
9	4052/32		3	7	163.0	23.3
10	3894/32	Nephrolithiasis	1	7	160.0	22.8
11	5664/32	Anaemia	1	7	135.0	19.3
12	5664/32		2	7	132.0	18.8
13	5664/32		3	7	137.0	19.5
14	5664/32		4	7	152.0	21.7
15	705/33	Carcinoma with metastases in bones	1	5	94.5	18.9
16	254/34	Anaemia	1	6	108.0	18.0
17	254/34		2	20	484.0	24.0
18	5240/35	Hyperthyroidism with osterporosis	1	5	119.0	23.8
19	5475/35	Syphilis: generalized decalcification of bones	1	6	152.1	25.3
20	5803/36	Coeliac disease	1	3	68.3	22.7

in the same individual in the different periods, but also in the different subjects. Statistically the following were the results:

	gm.
Arithmetical mean	21.5
Standard deviation	2.1
Probable error of the mean	0.31

It is quite possible that similar results may be obtained with other diets. The practical significance of the above observations, however, is that a fairly constant value has been found for the weight of dried feces under a given set of conditions. Aside, therefore, from its application in mineral balance experiments, this diet might also be found useful in studies of fecal weights in general.

LITERATURE CITED

RABINOWITCH, I. M. 1932 J. Nutrition, vol. 5, p. 325.

DESTRUCTION OF VITAMIN A BY RANCID FATS [1,2]

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INTRODUCTION

It has been known for some time that rancid fats destroy vitamin A. Fridericia ('24) and Powick ('25) found that the vitamin A in butterfat was destroyed when fed with very rancid fats. Nelson, Nelson and Lowe ('34) obtained similar results. They reported that enough rancidity was developed by fats when used as shortening to cause some destruction. Mattill ('27) demonstrated that both vitamins A and E were destroyed by rancid fats. Whipple ('36) found vitamin A to be destroyed in cod liver oil as rancidity developed. She related this destruction to factors of time and temperature as well as to the peroxide number of the fat. The work reported in this paper was undertaken to determine the extent of destruction [3] of vitamin A and carotene by fats of various degrees of rancidity as produced by heating, aeration and ozonization, and by admixture with various decomposition products.

METHODS

Attempts were made to determine the destruction of vitamin A in rations containing fat and fat-decomposition products by direct analysis using spectrophotometric and the

[1] The authors wish to express their appreciation of the financial support of the Lever Brothers Company, which made this work possible.

[2] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

[3] The term 'destruction' as here used refers to loss of ability to produce liver stores of vitamin A in the rat.

Carr-Price colorimetric technic. For this purpose the lipids of the ration were extracted, saponified, and the unsaponifiable matter chilled in methyl alcohol as outlined by Baumann and Steenbock ('33). Good recoveries of vitamin A were obtained from rations made with fresh fats, but with rancid fats or fresh fats to which aldehydes or ketones had been added, considerable difficulty was experienced. Variable spectral absorption was obtained at 328 μm, and the Carr-Price antimony trichloride reagent ('26) gave variable dark brown reaction mixtures.

Since several attempts to remove the interfering compounds chromatographically and phasically without affecting vitamin A values proved ineffective, the use of direct means of determination was abandoned, and an indirect method involving the feeding of the preparation to rats and the colorimetric determination of vitamin A in their livers was substituted. Rats 3 weeks of age with low vitamin A reserves (Baumann, Riising and Steenbock, '34) were used. After 5 weeks on the experimental diet the rats were decapitated, the livers removed, sliced and dissolved in 30 cc. of 8% potassium hydroxide at room temperature. Fifty-five cubic centimeters of water and 15 cc. of ethanol were added, and the mixture extracted repeatedly with peroxide-free ether. The ether was removed from the extract under reduced pressure, the fat was saponified and the vitamin A determined in the unsaponifiable matter colorimetrically by the Carr-Price technic. Whenever possible the operations were carried out in an atmosphere of nitrogen. The vitamin A content was calculated as outlined by Moore ('30).

The basal ration fed to rats was composed of crude casein 18, salts (Osborne and Mendel as modified by Hawk and Oser, '31) 5, brewer's yeast 8, sucrose 37, and fat 30. Unless otherwise indicated halibut liver oil [4] equivalent to 15 blue units of vitamin A per gram of ration was added. In the preparation

[4] The halibut liver oil was a commercial brand known as Haliver Oil furnished by The Abbott Laboratories.

of the rations the particular fat [5] or fat-decomposition product, when necessary, was melted at a low temperature and thoroughly mixed with the halibut liver oil. The mixture was allowed to stand for 5 minutes; then the other ingredients of the ration were incorporated. The rations were stored in a refrigerator at 5°C. and fed during the interval between the second and tenth day after preparation. The rats were fed daily, the food intake for all being kept the same, namely, 5 gm. per day the first week, 6 gm. the second, 7 gm. the third, and 8 gm. the fourth and fifth weeks. Six rats were usually used in each group. Analyses were made on each rat individually.

TABLE 1

Effect of rancidity of lard on storage of vitamin A

Peroxide number of fat fed	*Vitamin A per liver blue units* [1]
1	607 (516–602)
4	326 (220–440)
11	Traces
30	0

[1] Each value for blue units represents the average of four livers.

Rancid fats for routine use were prepared by aerating fresh fats with moist air at 75°C. with intense illumination from Mazda lamps. The peroxide number was determined iodometrically. It is expressed in terms of millimoles of oxygen per kilogram of fat.

RESULTS

Rancid fats. Pronounced destruction of vitamin A was found to take place with rancid fats but none with fresh fats. For example, with fresh lard, peroxide number of 1, good stores of vitamin A were obtained. When the peroxide number was increased to 4, the resultant storage was definitely less, and when the peroxide number was further increased to 11 or 30, only minimal amounts of vitamin A were stored (table 1). Similar results were obtained with a rancid hy-

[5] The hydrogenated fat was prepared commercially by hydrogenating cottonseed oil to an iodine number of approximately 70. The saponification number was 196.0. The lard used was a good quality, kettle-rendered product.

drogenated fat. In general the destructive action, paralleled an increase in peroxide number. A very rancid lard of a peroxide number of 200 was found capable of destroying 180 blue units of vitamin A per gram of ration.

Heated fats. In the processes of frying, roasting and baking, fats are subjected to high temperatures in the presence of oxygen, resulting in a certain amount of oxidation and decomposition. The effect of heated fats on vitamin A was, therefore, investigated. They were prepared by heating fresh fats for 1 hour in open pans at temperatures ranging from 100° to 200°C. at a depth of approximately 1 cm. Both lard and hydrogenated fat so treated were found to destroy vitamin A (table 2).

TABLE 2

Effect of heated fats on storage of vitamin A

TREATMENT OF FAT	LARD		HYDROGENATED FAT	
	Peroxide no.	Vitamin A per liver	Peroxide no.	Vitamin A per liver
		blue units [1]		*blue units* [1]
No treatment (fresh)	1.0	709	0.5	627
Heated 100°C. 1 hour	1.0	580	4.0	627
Heated 125°C. 1 hour	3.0	Trace	8.0	451
Heated 150°C. 1 hour	8.0	0	17.0	Trace to 120
Heated 175°C. 1 hour	18.0	0	10.0	Trace
Heated 200°C. 1 hour	8.0	0	8.0	Trace

[1] Each value for blue units represents the average of two animals.

The duration of the heat treatment as well as the temperature seemed to be factors in determining the resultant destructive capacity (table 3). With lard heated at 240° for 90 or 30 minutes, no liver stores of vitamin A were obtained, whereas when the time of heating was reduced to 5 minutes at 240°C., stores were again produced. In addition, it was found that as the amount of vitamin A administered was increased, complete destruction with the heated fat was no longer obtained.

Apparently 175°C. represented the optimum temperature for peroxide formation, and the use of higher temperatures resulted in lower peroxide numbers. Although the heated

fats with the higher peroxide numbers destroyed vitamin A, the destruction was not always proportional to the peroxide number. It was evident, however, that the destructive capacity induced by heating was the result of oxidation because after lard had been heated to 240° for 90 minutes in nitrogen, with no increase in its peroxide number, its effect on storage was almost the same as that of fresh lard. Specifically, from 473 to 588 blue units were stored on such treated lard as contrasted with 530 to 623 units on fresh lard.

Identity of destructive agents. In view of the role oxygen seemed to play in effecting destruction the effect of certain

TABLE 3

Effect of heated lard on storage of vitamin A

SERIES	VITAMIN A AS HALIBUT LIVER OIL PER GRAM OF RATION	TIME OF HEATING	TEMPERATURE OF HEATING	PEROXIDE NO.	VITAMIN A PER RAT LIVER
	blue units	*min.*	*°C.*		*blue units*
I	15	0	0	1	530–638 (5)
	15	5	240	2	367–400 (2)
	15	30	240	5	0 (6)
	15	90	240	6	0 (6)
II	30	60	185	12	0 (2)
	60	60	185	12	315–525 (2)
	120	60	185	12	955–998 (2)
III	30	120	240	6	Trace (2)
	60	120	240	6	861–955 (2)
	120	120	240	6	1260–1470 (2)

The numbers in parentheses give the number of rats used.

oxidizing compounds which might occur in rancid fats was tested. Palmitic peroxide was fed at a 3% level with 27% of fresh lard and 15 blue units of vitamin A per gram of ration. No vitamin A could be demonstrated in the livers. Ozonized lard,[6] peroxide number of 50, was also fed at a 5% level plus 25% of fresh lard, and again no vitamin A was stored even when the intake was increased to 50 blue units per gram of ration. Similarly, with oxidized oleic acid, peroxide number of 28, or with the oxidized fatty acids of lard,

[6] Ozonized lard was prepared by bubbling ozonized oxygen (containing 5% ozone) through melted lard until the peroxide number was 50.

peroxide number of 27, storage was not secured; but with fresh oleic acid fed as 10% of the ration, stores were readily obtained (table 4).

It was of interest to determine if rancid fats practically freed from peroxides could still destroy vitamin A. As it was found that the peroxide number of rancid fats could be materially reduced by heating at a high temperature in the absence of oxygen, a rancid hydrogenated fat, peroxide number of 100, was heated under a reflux condenser in an atmosphere of nitrogen at 165°C. for 1 hour to reduce its peroxide number to 4. This was fed with 25 blue units of vitamin A per gram of ration. From 385 to 660 blue units of vitamin A were stored. With fresh fat in a control experiment the stores ranged from 1310 to 1470 blue units. With the unheated rancid fat with a peroxide number of 100, no storage was obtained. It was evident from this that the destructive action was again quite out of proportion to the peroxide value.

An effort was made to remove the destructive agents from fats by direct extraction and by steam distillation. Heating rancid lard for 1 hour at 185°C. while passing live steam through it did not diminish its destructive properties. Also, extracting lard three times with three volumes of 95% alcohol failed to produce any effect. Furthermore, the volatile compounds removed by vacuum steam distillation following the commercial hydrogenation of fats were found to be nondestructive. Stores of 583 to 660 blue units were found to be produced when these latter compounds were fed at a level of 3% of the weight of the ration according to our usual technic.

The above results led us to turn our attention to the destructive effect of other possible constituents of rancid fats. As examples of aldehydes and ketones which might be present in rancid fats, chemically pure, straight-chain aldehydes and methyl ketones [7] containing 7 to 12 carbon atoms were mixed with fresh lard so that their final concentration in the ration was 0.1%. This gave a much higher concentration of such constituents than is found in an ordinary rancid fat as evidenced by the resultant strong odor after their incorporation.

[7] Furnished by Lever Brothers Company, Cambridge, Massachusetts.

TABLE 4

Destructive effect of miscellaneous materials

COMPOUND TESTED	AMOUNT OF COMPOUND IN RATION	AMOUNT OF FAT IN RATION	VITAMIN A PER GRAM OF RATION	VITAMIN A PER LIVER
	%	%	*blue units*	*blue units*
Allyl alcohol	0.05	30 fresh lard	15 [1]	567
Acrolein	0.05	30 fresh lard	15	555
Acrylic acid	0.05	30 fresh lard	15	574
Pyruvic acid	0.05	30 fresh lard	15	605
Propyl aldehyde	0.05	30 fresh lard	15	601
Glyceric aldehyde	0.50	30 fresh lard	15	506
Glyceric acid	1.0	30 fresh lard	15	567
Palmitic peroxide	3.0	27 fresh lard	15	0
Ozonized lard, per. no. 50	5.0	25 fresh lard	50	0
Oxidized oleic acid, per. no. 28	10.0	0	15	0
Oxidized fatty acids of lard, per. no. 27	10.0	0	15	0
Trilaurin	30.0	0	15	429
Hydroquinone	1% of fat	30 rancid, per. no. 33	15	0
Ascorbic acid	1% of fat	30 rancid, per. no. 33	15	0
Gallic acid	1% of fat	30 rancid, per. no. 33	15	0
Ethyl gallate	1% of fat	30 rancid, per. no. 33	15	0
$CH_3(CH_2)_{10}CHO$ [2]	0.1	30 fresh lard	2% cod liver oil	2870
$CH_3(CH_2)_9COCH_3$	0.1	30 fresh lard	2% cod liver oil	3198
Glycerol heated in light 48 hours at 100°C.[3]	30.0	0	15 [1]	544
Glycerol heated 40 hours, 100°C., CuO present	30.0	0	15	601
Fresh glycerol	30.0	0	15	583

[1] As halibut liver oil.

[2] C_7 to C_{11} aldehydes or ketones gave similar results.

[3] Glycerol heated with and without aeration at 170°C. and under reflux produced the same result.

Two per cent of cod liver oil was used in the ration as the source of vitamin A. Analysis of the resultant livers failed to reveal any evidence of a destructive effect (table 4).

Not only fatty acids but also glycerol was assumed to be a possible antecedent of destructive agents. Accordingly, glycerol was fed after being treated in various ways. It was heated under a reflux condenser at 185°C., heated and aerated at 170°C., aerated at 100°C. with intense illumination, and aerated at 200°C. in the presence of CuO. All of the resultant products were found to be inactive. Pure allyl alcohol, acrolein, acrylic acid, pyruvic acid, or propyl aldehyde at 0.05%, glyceric aldehyde at 0.5%, or glyceric acid at 1.0% of the ration were also found to be inert (table 4).

It is well known that rancid fats absorb oxygen rapidly because of their low content of substances which inhibit the oxidative processes. If the absence of antioxidants was the cause of more extensive destruction, then a synthetic fat should be especially destructive. To determine this, trilaurin was synthesized by heating glycerol with redistilled lauric acid and then incorporated at a 30% level together with halibut liver oil in the basal ration. The resultant storage of vitamin A was of the order obtained with a fresh hydrogenated fat (table 4).

As a corollary of the above experiment, certain antioxidants and easily oxidizable compounds, such as hydroquinone, ascorbic acid, gallic acid and ethyl gallate, were incorporated in rancid fats with a peroxide number of 33 and the resulting mixture tested for its ability to destroy vitamin A. These compounds were thoroughly mixed in the rancid fats, halibut liver oil was then added, and the resultant mixture incorporated in the basal ration. Practically no vitamin A was stored as the result of feeding these mixtures (table 4). When the experiment was repeated with lard of a lower peroxide value, namely 11, entirely similar results were obtained. As 11 is a low peroxide value for a rancid fat, it is evident that the antioxidants exerted but little, if any action over a protracted period.

Conditions necessary for destruction of vitamin A. From the data so far obtained it was not clear whether vitamin A was destroyed in the mixture of ration ingredients or after consumption by the animal. To determine if the vitamin had to be mixed with the rancid fat to bring about destruction, the two were fed separately at different times after the gastro-enteric tracts of the rats had been emptied by fasting. Fresh lard and lard heated at 240°C. for 90 minutes at a depth of 1 cm. were used as the fats in the rations. After the rats had been fasted for 15 hours, they were given 500 blue units of vitamin A as halibut liver oil by dropper. To facilitate administration, the liver oil was diluted with Wesson oil. No food was given until 6 hours after this administration. When the rats were killed, it was found that all had the same amount of vitamin A in their livers irrespective of whether they had received the fresh or rancid fat. An additional experiment showed that palmitic peroxide fed before a fresh fat ration produced the same results. The results with rancid fat confirm the experience of Powick ('25), which suggested that rancid lard had less effect on the destruction of vitamin A when the two were fed separately than when intimately mixed before feeding, but in his experiments the tract apparently was not cleared of rancid fat before the vitamin A was given, accounting for the possibility that some destruction still occurred.

It seemed possible that ingredients of the ration other than rancid fat might be causing the destruction of vitamin A when all were mixed. With this in mind, in another experiment, the basal ration was withheld from the rats for a period of 14 hours before and 8 hours after giving them 1 cc. of a mixture of rancid lard and vitamin A by stomach tube; this was done three times weekly. The rancid lards used had a peroxide number of 33 in one series, and of 240 in another. They were supplemented with 200 and 400 units of vitamin A respectively and kept under nitrogen. Control experiments were carried out with fresh lard and vitamin A fed in a similar manner. Storage of vitamin A was not obtained with

either of the rancid lards, but with fresh lard, averages of 179 and 937 blue units were obtained with weekly intakes of 600 and 1200 units of vitamin A respectively. It is thus apparent that the basal ingredients of the ration were not responsible for the destruction occasioned by rancid fats. Since the halibut liver oil and rancid lard were mixed and kept under nitrogen at all times, the effect of atmospheric oxygen was also minimized in these experiments.

Since it was necessary for the vitamin A to be mixed with the rancid fat to secure destruction, the question arose as to whether or not destruction could occur in the time interval necessary for digestion to take place in the gastro-enteric tract. This made it desirable to secure data on the stability of the peroxide of rancid fats under the conditions prevailing in the tract.

Normal rats weighing approximately 200 gm. were starved for 48 hours and then given by stomach tube 2 cc. of rancid fat of a peroxide number of 202. From time to time a few animals were decapitated, the stomachs removed, and the contents washed into a beaker. The fat was extracted with ether, filtered and the ether removed under reduced pressure in an atmosphere of nitrogen. The extracted fat removed from the stomach 2 minutes, 90 minutes or 4 hours after feeding was found to have practically identical peroxide numbers.

As the peroxide number of a rancid fat was but little changed in the stomach, the fat containing such peroxides should, therefore, be able to destroy vitamin A if given sufficient time. To determine the justifiability of this inference another experiment was performed in which vitamin A was mixed with rancid fats already in the stomach. To effect this, food was withheld from rats for 22 hours daily; they were then given the basal ration containing 30% of a rancid lard of a peroxide number of 33. During this latter period, a drop of a mixture of halibut liver oil and Wesson oil containing 50 blue units of vitamin A was given by dropper. Under these conditions, which limited the reaction time to that necessary for digestion and absorption, practically no

vitamin A was found to be stored; only a few rats stored very small amounts. Control rats given fresh lard and vitamin A in the same manner stored vitamin A in quantity.

Destruction of vitamin A in foodstuffs. In the practical evaluation of foods for man and many animals, carotene must be recognized as the most common source of vitamin A. For this reason stability experiments were carried out with crystalline carotene and with carotene as found in plant tissue such as alfalfa. Some experiments were also carried out with egg yolk as a food source of vitamin A. Lard and hydrogenated fat were rancidified to peroxide values of approximately 33 and fed with vitamin A provided respectively, as 25 micrograms of pure carotene per gram of ration, as 10% of hard-boiled egg yolk or 10% of alfalfa meal.

No stores of vitamin A were produced when rancid fat was fed, but with fresh fat, stores were obtained. Approximately 500 blue units per liver were produced by carotene, 100 to 160 units with egg yolk, and 125 to 160 units with alfalfa meal. Rancid fats, therefore, destroyed not only vitamin A in halibut liver oil but also vitamin A or its equivalent precursors in egg yolk and alfalfa meal, and carotene itself.

In view of the lability of vitamin A in its various forms, the pertinent question which remains to be answered from the practical standpoint is whether the degree of rancidity which develops in natural fats and foods is of an order which could cause destruction. To throw light on this, an experiment was carried out in which fats which had been used in the frying of doughnuts and potato chips and for making pie crust were tested for their destructive action. The foods themselves were kept for a number of days at an elevated temperature to provide an opportunity for the development of rancidity. The fats were then extracted and found to have peroxide numbers ranging from 1.4 to 106, varying with the kind of fat and the purpose for which it had been used.

It was found that some destruction of vitamin A resulted when the peroxide numbers attained a value somewhat greater

than 1. When the peroxide value had been increased to approximately 30, vitamin A was no longer stored. For practical purposes consideration must be given to the fact that with fats of a peroxide number of 30 or more the odor and taste of the food makes them rarely tolerable for human consumption unless the rancidity is masked by the characteristic flavors and aroma of the food itself. To what extent this degree of rancidity develops during the storage of food while awaiting sale and consumption remains to be established. Our experiments did not include determinations of this character.

SUMMARY

Rancid fats were found to destroy pure carotene and vitamin A or precursors thereof as contained in halibut liver oil, egg yolk, or alfalfa. Ozonized fats and palmitic peroxide also destroyed vitamin A.

Fats which had been heated at such temperatures as are frequently used in cooking were found to destroy vitamin A to some extent. Vitamin A was not destroyed by oxidized glycerol, glyceric acid, glyceric aldehyde, allyl alcohol, acrolein, acrylic acid, pyruvic acid, propyl aldehyde, straight-chain aldehydes and methyl ketones of 7 to 12 carbon atoms, or decomposition products formed in the commercial hydrogenation of fats or by fresh fats heated in the absence of oxygen.

The components of rancid fats which caused destruction were not removed by distillation with steam nor extraction with alcohol. Heating of rancid fats lowered both their capacity to destroy vitamin A and their peroxide value.

The addition to rancid fats of ascorbic acid, hydroquinone, gallic acid, and ethyl gallate as antioxidants did not prevent the destruction of vitamin A.

Vitamin A was not destroyed when given to rats on an empty stomach even though they were being maintained on a ration rich in rancid fat.

Peroxides of rancid fats were stable for as long as 4 hours in the stomach, and destruction occurred when vitamin A and rancid fat were combined here for the first time.

LITERATURE CITED

BAUMANN, C. A., AND H. STEENBOCK 1933 The carotene and vitamin A content of butter. J. Biol. Chem., vol. 101, p. 547.

BAUMANN, C. A., B. M. RIISING AND H. STEENBOCK 1934 The absorption and storage of vitamin A in the rat. J. Biol. Chem., vol. 107, p. 705.

CARR, F. H., AND E. A. PRICE 1926 Color reactions attributed to vitamin A. Biochem. J., vol. 20, p. 497.

FRIDERICIA, L. S. 1924 Inactivating action of some fats on vitamin A in other fats. J. Biol. Chem., vol. 62, p. 471.

HAWK, P. B., AND B. L. OSER 1931 A modification of the Osborne-Mendel salt mixture. Science, vol. 74, p. 369.

MATTILL, H. A. 1927 The oxidative destruction of vitamins A and E. J. Am. Med. Assoc., vol. 89, p. 1505.

MOORE, T. 1930 Vitamin A and carotene. Biochem. J., vol. 24, p. 692.

NELSON, V. E., P. M. NELSON AND B. LOWE 1934 The effect of hydrogenated lard, storage lard and heated lard on the destruction of vitamin A in foods. Iowa Agr. Exp. Sta. Report, p. 81.

POWICK, W. C. 1925 Inactivation of vitamin A by rancid fat. J. Agr. Res., vol. 31, p. 1017.

WHIPPLE, D. V. 1936 The destruction of vitamin A by rancid cod liver oil. Oil and Soap, vol. 13, p. 231.

THE LACK OF NERVE DEGENERATION IN UNCOMPLICATED VITAMIN B_1 DEFICIENCY IN THE CHICK AND THE RAT [1]

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INTRODUCTION

The nervous manifestations of beriberi, or vitamin B_1 deficiency, have been described frequently. There is lack of agreement among investigators as to whether acute vitamin B_1 deficiency causes degeneration of the nervous system.

Davison and Stone ('37), Zimmerman ('37), Lee and Sure ('37), as well as others, have demonstrated varying degrees of nervous system degeneration in B_1 avitaminosis of experimental animals. Beriberi in man is described in an extensive review (Cowgill, '32) as a multiple peripheral neuritis.

On the other hand, Kon and Drummond ('27), Prickett ('34), Moore, Plymate and Andrews ('32), and Grinker and Kandel ('33) failed to show degenerative changes in the nervous system caused by a lack of vitamin B_1 in experimental animals. Woollard ('27) found that starvation was in large part responsible for nerve changes in vitamin B_1 deficient chicks.

The lack of agreement among different workers suggests that the vitamin B_1 deficiencies studied varied in severity or were produced under quite different dietary conditions.

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

Arnold and Elvehjem ('38 a, b) developed a vitamin B_1 deficient ration for the rat and also one for the chick. These rations gave excellent growth when fortified with vitamin B_1 and were successfully used for biological assay. It seemed that such rations would afford an adequate supply of the various food factors and offer a means of studying uncomplicated vitamin B_1 neuropathology.

A series of histologic studies from animals raised on these diets have now been made. It has been demonstrated that certain dietary factors influence the development of nerve degeneration in the chick fed vitamin B_1 deficient diets, and that an uncomplicated deficiency of vitamin B_1 does not cause degenerative changes in the peripheral nerves and spinal cord.

Two sets of experiments have been made, one with the growing chick (part I) and the other with the growing rat (part II).

HISTOLOGIC TECHNIC

All experimental animals were sacrificed by bleeding through the carotid vessels. Tissues were obtained only when typical severe B_1 deficiency symptoms were in evidence. Control animals were simultaneously autopsied.

The sciatic nerve was removed and fixed in 10% buffered formalin (pH 7). Frozen sections of portions of the nerve were examined in plane polarized light between crossed nicols according to the method of Setterfield and Sutton ('35). This method was found to be unsuited for the study of the spinal cord and optic nerve. Other portions of the nerve were embedded in paraffin, sectioned and stained with osmic acid by the rapid Marchi method and also the Sudan III stain of Kawamura and Yasaki ('36). Positive degeneration was recorded when each of the three technics showed degenerative changes; when degeneration was noted by one or only two methods, the results were recorded as questionable. Sections of the spinal cord were stained with Sudan III and osmic acid. Axis cylinders were stained according to the method of Bodian ('36). Pieces of spinal cord, nerve and thin slices

of brain (2 to 3 mm.) were also fixed and dehydrated at low temperature according to the method of Scott and Williams ('36). These tissues were stained with Giemsa's stain. This method proved to be a valuable one for the study of cellular details. Zencker's fluid was used as a fixative for the cell studies in the brain, liver, kidney, pancreas, intestine and spinal cord.

EXPERIMENTAL

Part I

In this series of experiments, white leghorn chicks were employed. The vitamin B_1 deficient basal ration 242A used by Arnold and Elvehjem ('38 a) was employed to produce B_1 avitaminosis, and consisted of:

A. Autoclaved portion:	
ground yellow corn	57
wheat middlings	25
crude acid precipitated casein	12
B. Untreated portion:	
dried whole liver substance	1
iodized salt	1
$CaCO_3$	1
$Ca_3(PO_4)_2$	1
cod liver oil	2

This ration with none of its ingredients autoclaved constitutes ration 351 or the control ration. Day-old chicks placed on the vitamin B_1 deficient ration developed typical deficiency symptoms in 12 to 14 days. Feeding ration 351 for 10 days followed by feeding ration 242A produced the deficiency symptoms in 17 to 21 days.

To determine the effect of inanition, the food intake of chicks on the autoclaved ration was measured daily and a like number of chicks were given an equivalent amount of ration 351.

A series of seven experiments were first made to determine the effect of extra vitamin A supplements upon the nervous system pathology which accompanies inanition and vitamin B_1 deficiency. Each experiment consisted of three to four birds per lot because a small number of animals per cage facilitated the paired lot-feeding method indicated above.

For the sake of convenience, the dietary manipulation employed and the results of these experiments have been summarized in table 1. Percomorph oil or a water suspension of β-carotene served as the source of vitamin A. Several methods of administration were used. Percomorph oil was fed alone or diluted with Wesson oil, and the water suspension of β-carotene was given orally as well as injected intraperitoneally. All methods seemed to be effective.

TABLE 1

A summary of the occurrence of myelin sheath degeneration in the sciatic nerve of vitamin B_1 deficient chicks and the influence of dietary manipulation

GROUP	RATION	NUMBER OF CASES	POSITIVE DEGENERATION	QUESTIONABLE DEGENERATION	PER CENT POSITIVE CASES
Lot I	Control ration (351 normal)	15	0	4	0
Lot II	B_1 deficient ration	23	8	9	34.8
Lot III	B_1 deficient ration plus 400 micrograms of vitamin A or β-carotene/wk.	25	4	10	16.0
Lot IV	Ration 351—restricted intake	19	6	8	31.6
Lot V	Ration 351—restricted intake plus 400 micrograms vitamin A or β-carotene/wk.	19	6	12	31.6

It is seen from the data compiled in table 1 that the feeding of extra doses of vitamin A or β-carotene to vitamin B_1 deficient chicks considerably reduced the incidence of myelin sheath degeneration. These data further indicate that vitamin A deficiency was not the causative factor for the nerve degeneration observed in inanition.

In the Marchi preparations a typical change was observed in the spinal cords of some of the chicks on the restricted food intake or on the B_1 deficient diet. The sections were characterized by a very fine dust-like dispersion. This condition occurred in about 50% of the cases. Vitamin A or β-carotene

administration failed to prevent its appearance. Similarly prepared sections of spinal cords from the control animals were free from this condition. Therefore, this reaction would seem to reflect the nutritional state of the birds and not to be a casual artifact.

Next it seemed advisable to test the efficacy of a high level of fat in the B_1 deficient diet for alleviating B_1 deficiency symptoms and also to study the influence of added riboflavin in the development of a vitamin B_1 deficiency.

To study the effects of fat, five chicks were fed the vitamin B_1 deficient ration plus butter oil in capsules in amounts equivalent to 33% of their ration. All of these animals developed the typical B_1 deficiency symptoms earlier than did those fed the vitamin B_1 deficient ration alone. The sciatic nerves of these animals were normal and the spinal cords did not show the dust-like myelin reaction with osmic acid. Apparently the nutritive state of the spinal cord was improved by including a high level of butter oil in the diet.

Five more chicks were fed ration 242A (B_1 deficient) modified to contain twenty parts of yellow corn and twenty parts of glycerol. From previous experience it had been suggested that glycerol added to ration 242A might be more palatable for the chick than the same ration modified to contain butter oil. The replacement of the carbohydrate by glycerol was ineffective in protecting chicks from B_1 deficiency symptoms. Several cases developed myelin sheath changes in the spinal cords and sciatic nerves (table 2).

It seemed possible from the work of Phillips and Engel ('38) that the riboflavin content of the ration might also be involved in vitamin B_1 deficiency, especially since inanition always accompanies the latter. Chicks were fed ration 242A (B_1 deficient) supplemented respectively with vitamin A, or vitamin A and riboflavin. Controls were fed an adequate diet (ration 351) but restricted to a daily food-intake equivalent to the food ingested by the birds on the B_1 deficient diet. Here too, added amounts of vitamin A, or riboflavin and vitamin A were given. The riboflavin (Labco lactoflavin 1B)

was added to the daily ration in sufficient quantity to furnish at least 50 micrograms of riboflavin per chick per day in all cases. Percomorph oil was used as a source of the added vitamin A. The dietary regimen and results from these experiments are shown in table 2. The data show that no degenerative changes appeared in the myelin sheaths of the chicks when positive and adequate extra doses of vitamin A and riboflavin were fed. Where vitamin A was given alone degenerative changes were not infrequently evident in the myelin.

TABLE 2

The influence of riboflavin and vitamin A in the development of myelin degeneration in the chick on B_1 deficient diets

GROUP	RATION	NUMBER OF CASES STUDIED	CASES WITH POSITIVE DEGENERATION IN THE SPINAL CORD	CASES WITH POSITIVE DEGENERATION IN THE SCIATIC NERVE
Lot I	B_1 deficient ration modified to contain 20% glycerol	6	2	2
Lot II	B_1 deficient ration plus vitamin A[1]	6	0	1
Lot III	B_1 deficient ration plus vitamin A[1] plus riboflavin	8	0	0
Lot IV	Ration 351 plus restricted intake plus vitamin A[1]	6	1	1
Lot V	Ration 351 restricted intake plus vitamin A[1] plus riboflavin	9	0	0

[1] 400 micrograms per week.

Summary of pathology. The B_1 deficient chick ration when fed to day-old chicks caused degenerative changes of the myelin in the spinal cords and peripheral nerves of one-third of the cases. This neuropathology was very mild in character. Adequate positive extra doses of vitamin A and of riboflavin eliminated all signs of nerve pathology in the myelin sheaths. Thus, chicks with uncomplicated vitamin B_1 deficiency showed no signs of nerve degeneration.

No pathologic changes were observed in the axis cylinder in either B_1 avitaminosis or inanition. No histologic changes were observed in the brains of these chicks. Neither was

cellular changes noted in sections of spinal cords and dorsal root ganglia when fixed by the freezing-drying method and stained with Giemsa's stain. Other tissues, namely, kidney, liver and pancreas were apparently normal.

Part II

Histologic observations made in this laboratory over a period of 2 years indicated that nervous system pathology in vitamin B_1 deficiency in the rat was of very infrequent occurrence. To test this point more completely, an experiment was set up to study the neuropathology in the acute stage of deficiency and in recovery. Thirty young rats, weaned at 3 to 4 weeks of age were divided into three lots. All three lots were placed on vitamin B_1 deficient ration 112 of Arnold and Elvehjem ('38 b). This ration was composed of:

Sucrose		62
Purified casein		18
Autoclaved peanuts		10
Autoclaved A.B. yeast		4
Salts I		4
Factor W	≅	2 liver extract
Percomorph oil		3 drops weekly

The animals were kept on this ration for about 6 weeks at which time severe vitamin B_1 deficient symptoms had developed. The rats from lot I were sacrificed when severe symptoms were in evidence. The lot II animals, after severe symptoms had developed, were continued on the same ration plus 120 micrograms of crystalline vitamin B_1 per 100 gm. of ration. This group served as a positive control. The lot III animals, with severe vitamin B_1 deficient symptoms, were continued on the B_1 deficient diet modified so that the carbohydrate was isocalorically replaced by fat (Stirn and Arnold, '38). The 62% sucrose was replaced by 26% butter oil.

The animals in lots II and III were kept on the recovery diets for 1 week, then sacrificed and tissues removed for histologic examination.

The spinal cords and sciatic nerves from rats (lot I) which were sacrificed when severe vitamin B_1 deficient symptoms had developed were found to be normal in all cases when stained with Marchi. Only two cases out of twelve were diagnosed as possible degeneration in the sciatic nerve by the polarized light technic. The ten cases studied in lot II (recovery from severe symptoms with crystalline vitamin B_1) were diagnosed as having normal sciatic nerves and spinal cords. The animals (five cases only) in lot III (recovered by an isocaloric replacement of the carbohydrate of the diet with fat) all had normal sciatics and spinal cords. Three animals in this lot with severe symptoms failed to consume the high fat diet and were therefore autopsied with severe symptoms and histologic findings thus reported.

In view of Best and Huntsman's findings ('35), that high fat diets produce fatty liver unless sufficient choline was also present in the diet, the livers of the animals from lot III were removed for histologic examination. They were found to be normal in all cases. The livers from five animals in each of the other two lots were also studied. The animals with severe B_1 deficiency symptoms (lot I) had three cases with slight vacuolization of the liver cells. On the other hand, the livers from the animals fed crystalline vitamin B_1 (lot II) were all pathological. The histologic picture was characterized by cloudy swelling, and fatty degeneration. The latter was so extreme that staining of the cytoplasm was completely absent or nearly so. The nuclei were normal.

To investigate further this pathological change in the livers, six more cases were studied. These cases were injected with 500 gamma of vitamin B_1 at the time when severe B_1 deficiency symptoms were apparent. All rats were continued on the basal ration. Two were sacrificed 15 hours after B_1 administration, two were sacrificed on the third day and two on the seventh day. In these cases the pathology of the liver described above in recovery from B_1 deficiency was confirmed.

Summary of pathology. Histologic studies of acute but uncomplicated vitamin B_1 deficiency in the rat revealed

normal myelin in peripheral nerves and spinal cords. Other tissues studied in the deficiency condition, namely, brain, liver, kidney, pancreas and intestine were found to be free from any pathologic changes.

Rats in recovery from vitamin B_1 deficiency by crystalline vitamin B_1 administration showed a characteristic vacuolization, indicative of fatty degeneration in the liver cells. The metabolic disorder responsible for this condition is not clear. On the other hand, animals continued on the B_1 basal ration with an isocaloric replacement of the carbohydrate by fat presented a normal histologic liver structure.

DISCUSSION

These data demonstrate clearly that an uncomplicated vitamin B_1 deficiency can be produced in experimental animals through the use of proper diets. Further, the nervous system changes occurring in vitamin B_1 deficiency in the chick have been demonstrated to be due to an insufficiency of other dietary essentials. An adequate ration restricted to a low daily food intake resulted in histologic reactions comparable to those observed in the vitamin B_1 deficient chicks. Thus one is forced to conclude that the peripheral nerve and spinal cord changes found in vitamin B_1 deficiency are not due to a lack of vitamin B_1. Our results are in agreement with Browning ('31) who reports that inanition in birds produces a condition indistinguishable from beriberi and with Woollard's ('27) finding that starvation is in a large part responsible for nerve changes in B_1 deficient chicks.

Mellanby ('34) and others have demonstrated that peripheral nerve degeneration occurs in vitamin A deficiency. He reports that once an anatomical change in the central nervous system appears in this deficiency, the administration of large amounts of vitamin A are ineffective in restoring normal conditions in the nervous system. Zimmerman ('37) and others, recognized that the pathology of vitamin A deficiency is closely associated with the nervous system. Fearson ('36) stated that the cause of degenerative changes in nerve structure in beriberi were due to avitaminosis A. These reports,

together with our findings, emphasize the necessity of assuring adequate vitamin A intake on vitamin B_1 deficient diets in pathologic studies.

The fact that riboflavin administration to vitamin B_1 deficient animals was effective in removing nervous system pathology is of interest in view of the report of Phillips and Engel ('38). They found that severe myelin sheath changes occurred in chicks on riboflavin deficient diets before any gross manifestations of the deficiency were apparent.

These findings argue strongly against the accumulated data indicating that beriberi is accompanied by degeneration in the nervous system. Our findings demonstrate that the nervous symptoms of uncomplicated experimental beriberi are not due to any definite anatomical change in the nervous system.

The immediate recovery observed when vitamin B_1 is given to beriberi animals further supports this position. Vitamin B_1 therapy in beriberi brings about a response in a few hours, an improbable feat in the case of true nerve degeneration. Further, the recovery which takes place in beribri rats when the carbohydrate in the diet is isocalorically replaced by fat adds further proof to this argument.

These studies do not concur with those of Davison and Stone ('37), Zimmerman ('37), Lee and Sure ('37), as well as others who have demonstrated varying degrees of nerve degeneration in B_1 avitaminosis of experimental animals and who have attributed these changes to vitamin B_1 deficiency.

In medical practice nerve degeneration is undoubtedly encountered in cases diagnosed as beriberi. However, deficiency diseases in man are seldom limited to a single factor. The nerve degeneration encountered in human B_1 deficiency (beriberi) is probably due to a lack of dietary factors other than vitamin B_1. Strauss ('38) points out the therapeutic value of vitamin B_1 in the treatment of polyneuritic patients. But the beneficial results obtained with vitamin B_1 therapy in the clinic may be the restoration to normal ingestion, utilization and metabolism of dietary factors essential to nervous system integrity.

SUMMARY

1. The administration of β-carotene or percomorph oil together with riboflavin removed all evidence of pathology in the peripheral nerves of vitamin B_1 deficient chicks or chicks fed an adequate ration at a restricted level of intake.

2. Neuropathology was seldom observed in a rat on a vitamin B_1 deficient but otherwise adequate diet.

3. Recovery from B_1 deficient symptoms in the rat by the administration of crystalline vitamin B_1 produced a marked histologic reaction in the liver.

4. It is concluded from these studies that uncomplicated beriberi is not accompanied by peripheral nerve degeneration.

LITERATURE CITED

ARNOLD, A., AND C. A. ELVEHJEM 1938 a Studies on the vitamin B_1 requirements of growing chicks. J. Nutrition, vol. 15, p. 403.

———— 1938 b Studies on the vitamin B_1 requirements of growing rats. J. Nutrition, vol. 15, p. 429.

BEST, C. H., AND M. ELINOR HUNTSMAN 1935 The effect of choline on the liver fat of rats in various states of nutrition. J. Physiol., vol. 83, p. 255.

BODIAN, DAVID 1936 A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec., vol. 65, p. 89.

BROWNING, E. 1931 The vitamins. Monograph 1 of the Pickett-Thomson Research Laboratory, London, Bailliere, Tindall and Co.

COWGILL, G. R. 1932 Vitamin B_1 in relation to the clinic. J. Am. Med. Assoc., vol. 98, p. 2282.

DAVISON, C., AND L. STONE 1937 Lesions of the nervous system of the rat in vitamin B deficiency. Arch. Path., vol. 23, p. 207.

FEARSON, W. R. 1936 Nutritional factors in disease. Monographs of medical and surgical science, p. 106. William Heineman (Medical books) Ltd., London.

GRINKER, R. R., AND E. KANDEL 1933 Experimental vitamin (A, B_1, B_2, and B complex) deficiency. Histologic changes in the central nervous system. Arch. Neurol. and Psychiat., vol. 30, p. 1287.

KAWAMURA, R., AND T. YASAKI 1936 Über eine neue Fettfarbungsmethode. Centr. Allg. Path. u. Path. Anat., Bd. 64, S. 177.

KON, S. K., AND J. C. DRUMMOND 1927 LXXXVIII. The physiological role of vitamin B, part III. Study of vitamin B deficiency in pigeons. Biochem. J., vol. 21, p. 632.

LEE, J., AND B. SURE 1937 Avitaminosis XIX. Nerve degeneration in albino rats as studied by freezing-drying method and polarized light with deficiency of vitamin A or of vitamin B. Arch. Path., vol. 24, p. 430.

MELLANBY, EDWARD 1934 Xerophthamia, trigeminal degeneration and vitamin A deficiency. J. Path. and Bact., vol. 38, p. 391.

MOORE, C. U., H. B. PLYMATE AND B. J. ANDREWS 1932 Studies in the B vitamins. V. A study of myelin degeneration in the peripheral nerves of rats as associated with low vitamin B content of diet. Am. J. Physiol., vol. 102, p. 598.

PHILLIPS, P. H., AND R. W. ENGEL 1938 Neuromalacia associated with riboflavin low diets. A preliminary report. Poul. Sci. In press.

PRICKET, C. O. 1934 The effect of a deficiency of vitamin B_1 upon the central and peripheral nervous systems of the rat. Am. J. Physiol., vol. 107, p. 459.

SCOTT, G. H., AND P. S. WILLIAMS 1936 A simplified cryostat for the dehydration of frozen tissues. Anat. Rec., vol. 66, p. 475.

SETTERFIELD, H. E., AND T. S. SUTTON 1935 The use of polarized light in the study of myelin degeneration. I. The appearance and progress of degeneration after transection of the sciatic nerve in the white rat. Anat. Rec., vol. 61, p. 397.

STERN, F. E., AND A. ARNOLD 1938 The relation of dietary fat to the vitamin B_1 requirement of growing rats. J. Biol. Chem., vol. 123, no. 3, Proc. Am. Soc. Biol. Chem., 117.

STRAUSS, M. B. 1938 The therapeutic use of vitamin B_1 in polyneuritis and cardiovascular conditions; clinical indications. J. Am. Med. Assoc., vol. 110, p. 953.

WOOLLARD, H. H. 1927 The nature of the structural changes in nerve endings in starvation and in beriberi. J. Anat., vol. 61, p. 283.

ZIMMERMAN, H. M. 1937 Die histologischen veränderungen des nervensystems bei experimenteller avitaminose A, B (B_1) und G (B_2). Schweitzer Archiv. für Neur. und Psychiat., Bd. 39, S. 195.

THE RELATION OF GROWTH AND NUTRITION TO THE RETICULOCYTE LEVEL IN THE YOUNG RAT

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THREE FIGURES

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The reticulocytes in the blood of the rat make up over 98% of the erythrocytes at birth, decrease to about 25% at 21 days, and reach the adult levels of 1.5 to 3% at 70 days of age (Orten and Smith, '34). During the course of experiments in which a tryptophane deficient diet [1] was fed to young rats, it was noted that loss in weight in the animals was accompanied by a decrease in the reticulocyte percentage in the blood. When the diet was supplemented with tryptophane, there was a gain in weight and a return of the reticulocytes to normal (Alt, '32). Smith and Gross ('37) have reported somewhat similar results in young rats whose growth was stunted by feeding diets deficient in lysine or total food energy. These workers studied the changes in the reticulocyte count at weekly intervals only. A decrease in the reticulocytes in the blood has been observed in adult rats and rabbits during the loss in weight associated with acute inanition (Fisch and Emmel, '24; Yamaguchi and Suzuki, '32). The present study further illustrates the close relationship between growth and nutrition and the reticulocyte level in the blood of young rats.

PROCEDURE

Young albino rats of the Wistar strain were used as experimental animals. All blood samples were drawn from the

[1] Diet prepared according to Berg and Rose ('29).

tails. Reticulocyte preparations were made on cover slips containing dried brilliant cresyl blue. In order to insure complete staining of the reticulocytes, the blood was allowed to be in contact with the dye for 1 to 2 minutes before making the smears. Five hundred cells were counted on each of two cover slips. Erythrocyte counts were made with Bureau of Standards equipment.

The stock colony ration consisted of two-thirds whole wheat (containing 1% iodized salt) and one-third whole milk

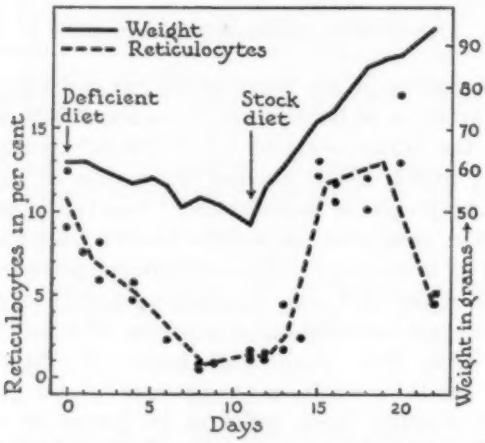

Fig. 1 The weights and reticulocyte counts of rats receiving first a diet deficient in tryptophane and later a stock diet. Weight curve represents average for the litter. Dots represent individual reticulocyte counts.

powder. Growing animals were stunted and made to lose weight either by feeding a diet deficient in an essential food substance or by restricting the quantity of the stock diet given. The deficient diet consisted of corn meal 30%, gelatin 10%, lactose 24%, cornstarch 19%, olive oil 8%, cod liver oil 2%, salt mixture 4%, agar 1.9%, cystine 0.1%, and yeast powder 1%. Unpublished work has shown that growth occurs when this diet is supplemented with tryptophane.

RESULTS

The effect of the diet deficient in tryptophane in a litter of five rats, age 33 days, is represented in figure 1. Coincident with the loss in weight, there was a decrease in the reticulocytes from a level of 10.9% to less than 1% on the eighth day of the experiment. When the stock diet was resumed, there was an immediate and rapid gain in weight. The reticulocytes, after a short lag, increased and reached a level of 12% within a period of 4 days. As the rate of gain in weight decreased, there was a secondary fall in the reticulocytes to normal levels.

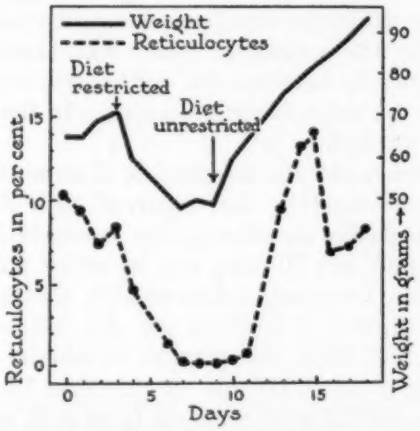

Fig. 2 The weights and reticulocyte counts of rats receiving first a restricted and later an unrestricted stock diet. Weight curve represents average for the litter. Dots represent individual reticulocyte counts.

In order to determine whether the decrease in the percentage of reticulocytes accompanying weight loss was related specifically to tryptophane deficiency, the same type of experiment was performed using a diet deficient in food energy (fig. 2). On consecutive days, restricted amounts of the stock diet (1, 2, 4, 5, 5 gm.) were given to a litter of four rats, age 37 days. The loss in weight was somewhat more rapid than in the preceding experiment, and the reticulocytes dropped from 8% to below 1% on the fourth day of restricted feeding. Realimentation on the sixth day was accompanied

by a rapid gain in weight, and the reticulocytes increased to a level of 13% within a period of 5 days. This was again followed by a secondary fall in these elements to normal levels. It seems then that the reduction in the reticulocytes accompanying weight loss was not related to a specific deficiency in the diet.

Determinations of the number of erythrocytes were performed along with the reticulocyte counts in the second experiment. During the loss in weight, two animals showed an increase of about 25% in the erythrocyte counts, whereas the remaining two showed no change. During the gain in weight and reticulocytosis accompanying realimentation, all animals had erythrocyte counts similar to the values obtained before the diet was restricted. There was, therefore, no consistent relationship between the erythrocyte counts and the variations in the reticulocyte percentages in the blood of the few animals studied.

A third experiment was designed to determine whether the decrease in reticulocytes that occurred with deficient diets was related to faulty nutrition in the hemopoietic system. A litter of six rats, age 70 days and weighing 115 to 120 gm., was divided into two groups of three each. Group 1 received a stock diet and group 2 received the diet deficient in tryptophane. After 25 days, the average weight in group 1 was 185 gm. and in group 2, 110 gm. At this time 1.5 cc. blood per 100 gm. body weight were removed from each rat by cardiac puncture, and the subsequent reticulocyte response studied. It can be seen in figure 3 that the reaction of the reticulocytes to an erythropoietic stimulus was only slightly less in the deficient animals than in the normal group. This indicates that the low reticulocyte counts in undernourished stunted rats were not primarily related to faulty nutrition in the hemopoietic system.

DISCUSSION

During the period of active growth in the rat, there is an increasing blood volume and, consequently, an increased demand for erythrocytes. This is reflected by the high percentage of reticulocytes in the blood. When growth is

stunted and loss in weight is produced by the feeding of inadequate diets, the blood volume decreases and the demand for erythrocytes is likewise decreased. A rapid fall in the reticulocytes was shown to occur during this period. Resumption of growth and improvement in nutrition which occurs with adequate feeding is accompanied by a sharp rise of the reticulocytes in the blood. The stimulus to erythropoiesis that occurs at this time and during normal growth is quite comparable to the stimulus that follows a single large bleeding. During growth the erythrocytes must keep pace with an increasing blood volume, while after bleeding a replacement of erythrocytes is necessary.

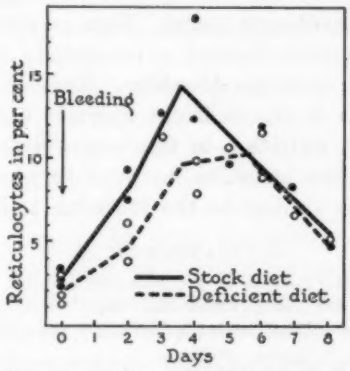

Fig. 3 The reticulocyte response to a single bleeding in rats receiving 1) a stock diet (dots) or 2) a diet deficient in tryptophane (circles).

Little is known about the nature of the stimulus that causes increased blood formation. Gordon and Dubin ('34) were unable to confirm earlier reports that a hemopoietic substance (Hemopoietine) may be found in the blood of animals rendered anemic or subjected to low pressures. Meyer, Stewart, Thewlis and Rusch ('37) reviewed the literature and reported experiments on the role of the hypophysis in hemopoiesis. They found that hypophysectomized rats subjected to low pressures did not show the usual reticulocyte rise seen in normal animals. Further, when hypophysectomized rats were given injections of pituitary growth hormone, there was a gain in weight and an increase in the reticulocytes, but there

was no change in the erythrocyte count and hemoglobin content of the blood. The authors recognized that this latter effect of the hypophysis on erythropoiesis might have been a non-specific one associated with growth. A better understanding of the nature of erythrocyte stimulating factors awaits future investigation.

SUMMARY

Young rats were given a diet either deficient in tryptophane or in total food energy. Loss in weight was accompanied by a rapid decrease in the reticulocyte level in the blood. With realimentation the gain in weight due to growth and improved nutrition was associated with a parallel increase in the reticulocyte count. Rats receiving the diet deficient in tryptophane showed a practically normal reticulocyte response to a single bleeding. This indicates that the reticulocytopenia in the deficient animals was not primarily related to faulty nutrition in the hemopoietic system. It is suggested that the stimulus to erythropoiesis that occurs during growth is similar to the stimulus following bleeding.

LITERATURE CITED

ALT, H. L. 1932 The relation of the reticulocyte count to tryptophane deficiency in the young albino rat. Read by title before the Central Society for Clinical Research, November 4th. (Abstract in program, p. 25.)

BERG, C. P., AND W. C. ROSE 1929 Tryptophane and growth. I. Growth upon a tryptophane-deficient basal diet supplemented at varying intervals by the separate feeding of tryptophane. J. Biol. Chem., vol. 82, p. 479.

FISCH, M. E., AND V. E. EMMEL 1924 Reduction in the percentage of erythrocytic reticulation and polychromasia in acute inanition. Anat. Rec., vol. 27, p. 181.

GORDON, A. S., AND M. DUBIN 1934 On the alleged presence of 'Hemopoietine' in the blood serum of rabbits either rendered anemic or subjected to low pressures. Am. J. Physiol., vol. 107, p. 704.

MEYER, O. O., G. E. STEWART, E. W. THEWLIS AND H. P. RUSCH 1937 The hypophysis and hematopoiesis. Folia haemat., Bd. 57, S. 99.

ORTEN, J. M., AND A. H. SMITH 1934 The proportion of reticulocytes in the blood of albino rats. Am. J. Physiol., vol. 108, p. 66.

SMITH, A. H., AND G. D. GROSS 1937 The relation of somatic growth to the maturation of the erythrocyte in the rat Am. J. Physiol., vol. 118, p. 309.

YAMAGUCHI, A., AND Y. SUZUKI 1932 Reticulocytes in normal and pathological conditions. II. Changes caused by hunger and bleeding. J. Orient. Med., vol. 17, p. 9.

NUTRITIONAL EFFECTS OF THE ADDITION OF MEAT AND GREEN VEGETABLE TO A WHEAT-AND-MILK DIET. EXPERIMENTS WITH RATS

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ONE FIGURE

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In feeding experiments the experimental variables may be of two kinds: 1) the individual chemical factors—elements or compounds as the case may be—or 2) the actual articles of food which nature and agriculture produce and which people and other animals consume.

In previous papers we have described improvements of already normal nutrition which were induced by a more scientific adjustment of the quantitative proportions of the articles of food in the dietary and have shown that calcium, vitamin A, and riboflavin was each an influential factor in these improvements (Sherman and Campbell, '24, '28, '30, '35 b, '37).

In the experiments here discussed the new variable was diversification, in terms of natural articles of food. To the more favorably constituted of the wheat and milk mixtures previously used, two such different foods as lean beef and a fresh green vegetable were added. This may be interpreted as a test of the effect upon nutritional well-being of the factor of diversification as such. And the finding, that the effect is so limited, may also be construed as an indication that whatever nutritional essentials still unrecognized there may be in such foods as muscle and as succulent green vegetable, are not significantly influencing the outcome of experiments in

normal nutrition planned and interpreted in terms of the factors now known; and therefore that it is scientific to make full practical use of present knowledge without awaiting further research upon 'new' nutritional factors.

EXPERIMENTAL

Diets. Two diets were used: 1) diet B (laboratory no. 13) consisting of whole milk powder 32.9%, ground whole wheat 65.8% and sodium chloride 1.3%; and 2) diet 138, consisting of this same dry food mixture fed ad libitum with the addition of meat (lean beef, top round, free from visible fat) at a level of 5 gm. per rat on each of 3 days per week, and green string beans (tips and stems removed) fed at the same level on the 3 alternating days, neither being fed on Sunday. The meat was obtained, freshly cut, each morning on which it was to be fed, and the beans were bought each day in the open market.

Except that the young rats did not always eat the full amount of meat for the first few days, the meat and beans were consumed very soon after being fed, while the dry diet was before the rats at all times.

Table 1 (a) gives the composition of diet B (diet 13) and of the meat and beans. As the amount of meat and beans was fixed but the animal was allowed to eat as much of the dry diet as it wished, only an approximate composition of diet 138 can be given, this approximation being made from a study of the food records at different ages. In the early period (fifth to eighth week of life, inclusive) the ratio of dry diet to meat and beans (in grams) was about 5:1:1; during youth and adult life (10 weeks to 2 years or more) it was 5.5 or 6:1:1; while in old age (850 or 900 days) if often dropped again to 5:1:1. An approximation of the amount of protein and other factors in diet 138 using this ratio is given in table 1 (a) but as diet 13 was a dry mixture while the beans and meat were fed in their natural moist form, and as the study of the food records showed that the rats receiving diet 138 ate in general as much of the dry diet as those receiving that alone, it seemed advisable to approach this problem from another angle also, namely, caloric value.

The caloric intake per gram of rat was the same on the two diets, and the ratio of calories from the dry diet to that from meat and beans in diet 138 was, in round numbers, 550 : 37 : 10. Therefore the approximate grams of protein, calcium and other factors in each 100 calories of diet are also given in table 1 (b) and will be referred to later.

Experimental procedure. In accordance with the method used in the previous experiments to which reference has been made, parallel lots of rats, each lot consisting of two males

TABLE 1

Composition of diets

	PROTEIN	CALCIUM	PHOSPHORUS	IRON	CALORIES	VITAMIN A VALUE	RIBOFLAVIN (VITAMIN G)
	%	%	%	%	*per gram*	*units per gram*	*units per gram*
a. Diet 13	15.42	0.348	0.495	0.00253	4.00	6.0	2.30
Lean beef	21.3	0.013	0.204	0.0030	1.56	0.66	0.6
String beans	2.3	0.054	0.049	0.00116	0.42	9.9	0.9
Diet 138[1]	14.53	0.264	0.424	0.00241	3.2	5.8	1.89
b. Approximate intake per 100 cals.[2]							
	gm.	*gm.*	*gm.*	*gm.*	*calories*	*units*	*units*
Diet 13	3.855	0.0869	0.1239	0.00063	100	150	57.5
Diet 138	4.492	0.0828	0.1242	0.00075	100	180	59.0

[1] Based on the ratio of average gram intake of dry diet and supplements as explained in the text. Diet 13 : meat : beans = 5.5 : 1 : 1.

[2] Based on the ratio of average intake of dry diet and supplements per 100 calories as explained in text. Diet 13 : meat : beans = 550 : 37 : 10.

and three females, were made up when the rats were 28 days old, the rats in one lot being litter mates of those in the other, and closely matched in weight.

Ten paired lots were thus started, the members of these lots being referred to as 'first generation' animals. A certain number of the offspring of these lots was continued on the parents' diet, these being selected 1) at different seasons of the year to avoid undue seasonal influence, 2) from litters of average weight for the diet, 3) from litters reared when the females were probably in their prime (that is, while some first

litters were kept, most were from second or third litters), and 4) at such a time and from such females that the later generation lots would be near enough of the same age and closely enough related to preserve the parallelism of the first generation lots.

All the experimental animals, either of the first or later generations were kept until death occurred from natural causes. The numbers on diet 13 were 70 males and 103 females; on diet 138, 69 males and 105 females.

TABLE 2

Summary of experimental data

	DIET 13			DIET 138			
	Number of cases	Gain in grams ±P.E.	Coef. of variation	Number of cases	Gain in grams ±P.E.	Coef. of variation	DIFFERENCE ± ITS P.E.
a. Growth data [1]							
Gain from 28th to 56th day of life							
Males	67	91.3 ±1.18	15.8	67	110.0 ±1.03	11.6	18.7 ±1.57 [2]
Females	100	68.5 ±0.53	11.6	94	79.3 ±0.59	10.8	10.8 ±0.79 [2]
Calories per gram of rat per day 5th to 8th week	27 lots [3]	0.41 ±0.004	7.8	32	0.41 ±0.005	6.3	0.004 ±0.0061
Gain per 1000 calories 5th to 8th week	27 lots	78.6 ±1.06	10.5	32	84.2 ±0.94	9.5	5.6 ±1.42 [2]
Gain per gram protein 5th to 8th week	27 lots	2.04 ±0.028	10.5	32	1.80 ±0.019	8.9	0.24 ±0.033 [2]

[1] In a few instances the animals were held on another diet until slightly more than 28 days of age, hence the number of cases reported here are slightly fewer than in data following.

[2] Indicates significant difference.

[3] Two males and three females in each lot.

TABLE 2—*continued*

	DIET 13			DIET 138			DIFFERENCE ± ITS P.E.
	Number of cases	Age in days ±P.E.	Coef. of variation	Number of cases	Age in days ±P.E.	Coef. of variation	
b. Breeding data							
Age at birth of first young	100 [2]	110± 1.2	16.2	104 [2]	103± 1.3	19.8	7± 1.7 [1]
Duration, breeding period	103 [2]	332±10.2	46.9	106 [2]	340±10.49	47.2	8±14.6
		Young per female ±P.E.			Young per female ±P.E.		
Number of young born	103 [2]	40.3±1.41	53.2	106 [2]	42.8±1.38	49.3	2.5±1.97
Number of young reared	103 [2]	27.0±1.10	62.1	106 [2]	31.9±1.19	57.7	4.9±1.62
Per cent of young reared	100 [2]	64.7±1.69	39.1	104 [2]	69.6±1.48	32.5	4.9±2.24
		Grams ±P.E.			Grams ±P.E.		
Average weight of young at 28 days	2784	49.4±0.05	8.2	3377	52.3±0.04	7.0	2.9±0.07 [1]
Average weight of male young	1439	50.5±0.15	16.4	1639	54.2±0.14	15.4	3.7±0.20
Average weight of female young	1412	48.3±0.13	15.7	1725	50.5±0.12	15.2	2.2±0.18
Remarks	9 (8.7%) reared no young. Of these 3 (2.9%) bore none, 6 others failed to rear ones born.			7 (6.7%) reared no young. Of these 2 (1.9%) bore none, 5 others failed to rear ones born.			
c. Longevity							
Length of life		Age in days ±P.E.			Age in days ±P.E.		
Males	70	724±10.9	18.8	69	722±10.9	18.8	2±15.4
Females	103	827±10.3	19.0	105	799±10.4	20.0	28±14.7
Number dying under 1 year		%			%		
Males	1	1.4	...	1	1.5	...	
Females	1	1.0	...	1	0.95	...	
Number living beyond 730 days							
Males	31	44.3	...	31	44.9	...	
Females	82	79.6	...	75	71.4	...	
Number living beyond 900 days							
Males	6	8.6	...	7	10.3	...	
Females	35	34.0	...	37	35.2	...	

[1] Indicates significant difference.

[2] Number of females in breeding lots.

[3] Number of females bearing young.

The quantitative results of the experiment are given in table 2 and figure 1. These findings may be discussed under the headings of 1) rate and efficiency of growth and development, 2) adult vitality, and 3) length of life.

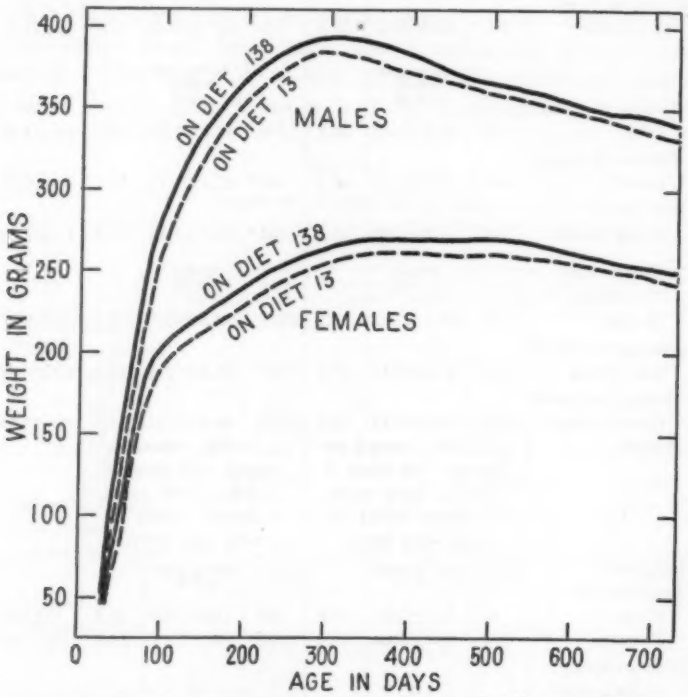

Fig. 1 Weight curves of rats on diets 13 and 138, respectively.

DISCUSSION

Rate and efficiency of growth and development. From figure 1 and table 2 (a) it will be seen that adding meat and green beans to the dry diet increased the rate of growth for both sexes as measured by the gain during the fifth to eighth week of the life of the rat; this increase in growth was statistically significant, the difference being about twelve times its probable error for each diet. The efficiency of growth on diet 138

as measured by gain per 1000 calories was significantly greater, but not as measured by gain per gram of protein. The food intake measured as calories per gram of rat was the same on the two diets at this period of growth as well as later, but referring to table 1 (b) it is seen that a larger per cent of the calories on diet 138 came from protein and also that each 1000 calories of diet 138 contained slightly more iron, possibly more vitamin A value; but practically the same amount of calcium, phosphorus and vitamin G as 1000 calories of diet 13. Some as yet undetermined substance in the meat and beans may have specifically stimulated growth or appetite; or the diversification of diet may in itself have had the effect of inducing the young animals to eat more. The latter is equally probable and a preferable hypothesis in that it refrains from postulating any new substance.

The rate of development as shown by the age at which the females bore their first young was very slightly but perhaps significantly increased, the difference being only 7 days, but this was four times its probable error.

Adult size and vitality. Figure 1 shows that the animals receiving the meat and beans not only grew more rapidly during the early period but attained a greater adult size and maintained this up to old age, the growth curves being nearly parallel after 84 days of age. In extreme old age (800 days and over) the survivors are few and individual variations are greater. Up to that time the coefficients of variation from the mean weights of the males at various ages were very uniform on both diets, about 10 to 11. On account of pregnancies it was impracticable to determine these for the females; but the coefficients of variation for the average weights of the females before maturity and after breeding ceased, were a little smaller than for the males. The food intake during the period of adult life was essentially the same for the two diets.

Similar short studies were made several years ago with larger amounts of beef and beans, liver in place of beef, lettuce in place of beans, or beef alone as supplements to diet 13.

With each of these types of diversification, growth was increased more when meat and vegetable were fed together than with meat alone.

The numerical data used as the criteria for adult vitality are 1) the time of maturity of the females as shown by their age at the birth of the first young, 2) the length of the period during which the females were able to bear and rear young, and 3) the number of young reared and their weight at 28 days of age. The findings are summarized in table 2 (b) and show that females on diet 138 matured earlier, and reared young which were significantly heavier at 28 days of age, this difference in weight being about the same for males and females. There were very few females on either diet that failed to bear and rear some young, but slightly fewer on diet 138. A slight but hardly significant difference in duration of breeding period, number of young born and reared and per cent of young reared was consistently in favor of diet 138. The same general trend was observed in the earlier experiments referred to on the previous page.

The appearance of the rats and the time of first signs of senility were recorded after frequent and careful observations. Parallel lots were observed simultaneously and the conditions recorded comparing one lot directly with the other. In many cases the protocol record was "no difference apparent between the two lots." In five cases, animals in the diet 138 lot were reported in better condition than those in the comparable diet 13 lot; in four cases, in poorer condition. Thus these two diets supported equally good apparent health. The average age at which the first signs of senility were reported was very nearly the same on the two diets, and for the two sexes, very slightly greater for the males on diet 138 and for the females on diet 13. Many of the animals on both diets remained in good condition up to 2 years. There was more variation in the time of onset of senility between animals on the same diet than between brother or sister rats on these two different diets.

A study was also made of the hemoglobin content of the blood of the rats on these two diets in connection with her study of rats on other diets by Doctor Ellis of this department (Ellis and Bessey, '35). She examined the blood from twelve males and eleven females on diet 138 and twelve males and twelve females on diet 13 at 1 month of age; and ten males and twelve females on diet 138, eight males and nine females on diet 13 at 1 year of age. At 1 month the hemoglobin of the males on diet 138 was slightly lower than for the males on diet 13, practically the same for the females on the two diets, but at 1 year of age the hemoglobin content of the blood of both males and females on diet 138 was slightly greater than for those on diet 13. Examination of the blood at 60 and 90 days showed that at 60 days both sexes on diet 138 had slightly surpassed those on diet 13 in hemoglobin content.

Length of life (table 2, c). The diversification of the diet had no significant influence upon the length of life. The females receiving the supplements died at a slightly earlier average age than those receiving only diet 13, but the difference is not statistically significant. The average age at death of the males on the two diets is practically the same, hence it is evident that the more rapid early growth, and attainment of a greater adult size by males on diet 138 did not decrease their life expectancy. Analysis of the data further shows no difference in the per cent of animals dying under 1 year or attaining an age of 2 years, or of 900 days, when the rats of the same sex are compared; but, as always in our colony, the females lived significantly longer than the males on the same diet, and a larger per cent lived to 900 days.

Over 100 animals on each diet were examined after death by Doctor Wilens or Doctor Sproul of the department of pathology of the College of Physicians and Surgeons. No definite correlation was found between diet and cause of death.

SUMMARY AND CONCLUSIONS

The complete life cycles were studied in 70 and 69 males and 103 and 105 female rats on two comparable diets, one differing from the other by the addition of fresh meat and green beans to the dry diet. The criteria which were used afforded measures of the efficiency of the diets in relation to growth and development, adult vitality, and length of life.

The addition of fresh meat and green beans was found to result in more rapid early growth, attainment and maintenance of larger adult size, and earlier maturity. It also prolonged very slightly the period of adult vitality as measured by the ability of the females to bear young. It did not significantly influence the length of life.

The cooperation of the Carnegie Corporation of New York and the Carnegie Institution of Washington is gratefully acknowledged.

LITERATURE CITED

ELLIS, L. N., AND O. A. BESSEY 1935 The effect of diet on the hemoglobin concentration of the blood. Am. J. Physiol., vol. 113, p. 582.

SHERMAN, H. C., AND H. L. CAMPBELL 1924 Improvement in nutrition resulting from an increased proportion of milk in the diet. J. Biol. Chem., vol. 6, p. 5.

———— 1928 The influence of food upon longevity. Proc. National Acad. Sci., vol. 14, p. 852.

———— 1930 Further experiments on the influence of food upon longevity. J. Nutrition, vol. 2, p. 415.

———— 1935 a Rate of growth and length of life. Proc. National Acad. Sci., vol. 21, p. 235.

———— 1935 b Effects of increasing the calcium content of a diet in which calcium is one of the limiting factors. J. Nutrition, vol. 10, p. 363.

———— 1937 Nutritional well-being and length of life as influenced by different enrichments of an already adequate diet. J. Nutrition, vol. 14, p. 609.

THE UTILIZATION OF CALCIUM [1]

FREDERICK F. TISDALL AND T. G. H. DRAKE

WITH THE TECHNICAL ASSISTANCE OF RUTH HERBERT

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ONE FIGURE

(Received for publication July 19, 1938)

The dietary calcium needs of children are usually regarded as at least 1 gm. of calcium per day. During adult life, after the bones and teeth are fully formed, the daily amount required is less than this. The most widely used figure for the calcium requirements of adults is that of Sherman ('32) of 0.68 gm. per day. In a recent study, Leitch ('37) gives a figure in fairly close agreement, namely, 0.55 gm. per day. During pregnancy and lactation, however, these needs are markedly increased so that at least 1.5 gm. of calcium are required daily to insure calcium equilibrium.

In view of these comparatively large amounts of calcium required for optimum health and the scarcity of this mineral element in practically all foods ordinarily consumed, with the exception of milk and milk products, the question of the availability of calcium in different forms and in different foods is an important one. With this in mind, it was decided to test the availability of calcium in different forms including those compounds used for therapeutic purposes. The rat was used as the experimental animal.

The total amount of calcium and phosphorus in the bodies of approximately 3-week-old rats with the intestines discarded, ranging in weight from 46 to 62 gm., was determined. The results on twenty-seven of these animals are given in figure 1.

[1] Read at the Sixteenth Annual Meeting of the Canadian Society for the Study of Diseases of Children, held at Kingston, Ontario, June 3 and 4, 1938.

This shows that there are variations not only according to the weight of the animal but also in different animals of the same weight. In an attempt to minimize these and other variations in the feeding observations to follow, the animals were matched according to weight, sex and litter. Six or more animals were used with each form of calcium tested.

The average total calcium and phosphorus in the bodies of 3-week-old animals, with the intestinal tract discarded, is

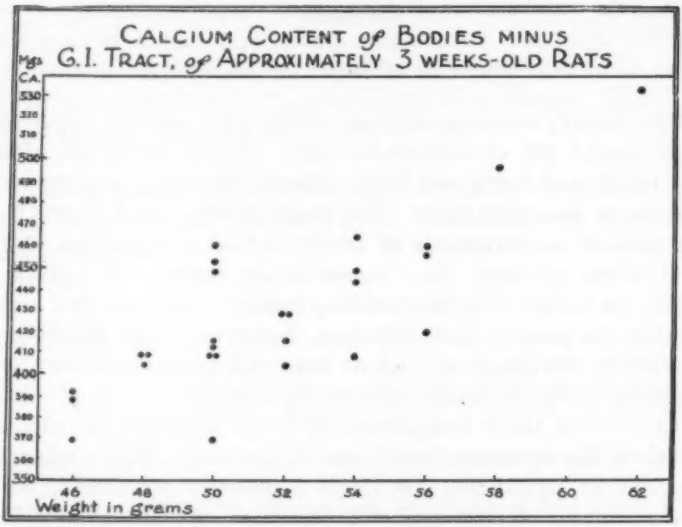

Figure 1

shown in table 1. A series of litter mates of these animals were fed for a further 4 weeks on the normal stock diet of the colony. These animals were then killed and the body calcium and phosphorus determined. A second matched series were fed 4 weeks on a low calcium but otherwise normal diet devised by Fincke and Sherman (see protocols). A third matched series was fed for 4 weeks on a low calcium diet devised by E. Chant Robertson (see protocols). The only deficiency in this diet, as in the case of Fincke and Sherman's

diet, was the low calcium. The phosphorus and other elements were normal. This diet contained 2% cod liver oil. The animals in these two series were also sacrificed after 4 weeks and the body calcium and phosphorus determined. The results are given in table 1.

Protocols

Fincke and Sherman's low calcium diet:

	%
Whole wheat	65.0
Skim milk powder	11.0
Butterfat	9.0
Cornstarch	13.7
Sodium chloride	1.3

E. Chant Robertson's low calcium diet:

Casein A and B free (alcohol and acetic acid extracted)	18.0
Cornstarch	62.2
Crisco	10.0
Yeast	6.0
Cod liver oil	2.0
Salt mixture	1.8

Salt mixture:

K_2HPO_4		219.72
KCl		125.29
NaCl		77.41
$MgCO_3$		33.43
$MgSO_4$ (anhydrous)		38.50
Fe Citrate $1\frac{1}{2}$ H_2O	94.18	13.80
NaF	3.68	
$MnSO_4$	1.17	
$K_2Al_2(SO_4)_4 \cdot 24\ H_2O$	0.67	
KI	0.30	
	100.00	

Diet for 17-year-old child with milk omitted:

Breakfast:	Luncheon:	Dinner:
Orange juice	Cold lamb	Clear soup
Cornflakes and cream	Potato and lettuce salad	Beef and potato
Bacon and egg	Canned peaches	Cabbage and peas
Toast and honey	Bread and butter	Apple pie
Coffee and cream	Plain cake	Bread and butter
	Tea and cream	Coffee and cream

From the results given in table 1, it is seen that Chant Robertson's diet resulted in a much lower retention of calcium than Fincke and Sherman's low calcium diet; consequently, the rats fed Chant Robertson's low calcium diet should be more sensitive to any calcium addition. For this reason, in all the subsequent experiments this diet was used as the basic low calcium diet.

Six litters of rats, each animal weighing approximately 50 gm., and each litter containing six animals of the same sex, were divided into six groups so that each group was matched with the other groups according to litter, sex and weight. To the first group was fed the low calcium diet. To the other groups was fed the same low calcium diet with the addition

TABLE 1

Average body calcium and phosphorus of rats fed different diets

DIET	WEIGHT AT 3 WEEKS	WEIGHT AT 7 WEEKS	Ca	P
	gm.	*gm.*	*mg.*	*mg.*
Normal	52	...	430	267
Normal	53	148	1406	780
Fincke and Sherman's low Ca	53	137	968	592
E.C.R. low Ca	51	116	482	392

of varying amounts of calcium in the form of calcium carbonate. The animals ate the diet ad lib., careful records being kept of the amount of food consumed by each individual rat. At the end of 4 weeks on the diets the animals were killed, the gastro-intestinal tracts discarded, and the total calcium and phosphorus in the animals' bodies determined. The difference between the amount of calcium and phosphorus in the bodies of the animals on the low calcium diet and the amount in the bodies of the animals with calcium added to this diet will be due to retention of the added calcium.

Table 2 shows that as extra calcium was added to the diet the calcium retention increased. A striking feature, however, was that as increased calcium retention occurred the amount of phosphorus retained also increased, although no phosphorus had been added to the diet. The ratio of the calcium

and phosphorus retention closely approximates 2:1. This is of particular interest, as it indicates that in a diet adequate in phosphorus but low in calcium the retention of the phosphorus is dependent on the retention of calcium.

The availability of calcium in the form of calcium carbonate, calcium chloride, dicalcium phosphate, calcium lactate, powdered whole milk, casec and Pablum was studied by adding 200 mg. per cent calcium in these various forms to the

TABLE 2

Extra Ca and P retention on addition of $CaCO_3$ to low Ca diet

Ca ADDED TO DIET	EXTRA Ca RETAINED	RETENTION EXTRA Ca FED	EXTRA P RETAINED	RATIO OF EXTRA $\frac{Ca}{P}$ RETAINED
%	*mg.*	%	*mg.*	
0.115	306	85	185	1.65
0.150	355	81	185	1.92
0.200	454	73	228	1.99
0.250	595	74	308	1.93
0.300	755	79	354	2.13

TABLE 3

Extra Ca and P retention on addition of 0.200% Ca in different forms to low Ca diet

FORM	EXTRA Ca RETAINED	RETENTION EXTRA Ca FED	EXTRA P RETAINED	RATIO OF EXTRA $\frac{Ca}{P}$ RETAINED
	mg.	%	*mg.*	
Dica. phosp.	467	77.2	206	2.27
Ca carb	454	74.0	228	1.99
Ca chlor.	470	74.7	215	2.19
Ca lact.	508	76.6	227	2.24
W. milk powder	459	72.9	249	1.84
Casec	458	77.6	233	1.97
Pablum	392	74.3	178	2.20

diet of rats for 4 weeks. The results given in table 3 show that the percentage retention of the extra calcium fed is approximately the same with all these different forms.

The various salts of calcium just studied were also fed at the level of 150 mg. per cent and 115 mg. per cent. Again the percentage of calcium retained was essentially the same with all these calcium salts. In these last series, calcium gluconate

was also fed and the amount of calcium retained did not differ from the other salts.

An attempt was made to estimate the availability of calcium in a number of vegetables, as prepared and canned for infant feeding. For practical reasons, it was necessary to feed the vegetables in dried form.[2] Due to the difficulties of drying these vegetables, it was necessary to add to the vegetables a certain amount of starch. In each animal experiment, the amount of dried vegetable added was such that the animal's diet contained the equivalent of 70 parts per 100 of the canned sieved vegetables undergoing test. Due to the fact that calcium is present in most vegetables in comparatively small amounts, the added calcium in most instances was comparatively small in relation to the control low calcium diet test. For this reason, no definite statement is made at the present time as to the availability of the calcium in the vegetables, except that there is some evidence that the calcium in vegetables does not seem to be utilized as efficiently as that of milk.

In another series of experiments, 10% dried spinach was added to the rat's diet, which is equivalent to about 210 parts of fresh spinach. At the same time, in a second series of animals, 115 mg. of calcium, which is the calcium content of 10 gm. of dried spinach, was added as calcium carbonate to each 100 gm. of the low calcium diet. To the diet of a third series of animals was added exactly the same amount of calcium carbonate, plus the amount of oxalic acid calculated to be present in the 10% dried spinach.

The results obtained, which are given in table 4, confirm the results previously reported by us (Tisdall, Drake, Summerfeldt and Jackson, '37) that the feeding of large amounts of spinach actually decreases the calcium retention. These results also show that this adverse effect of spinach on calcium retention is probably due in part at least to its oxalic acid content.

The effect of feeding 10% of the diet in the form of farina, oatmeal and Pablum on the calcium retention was studied.

[2] We are indebted to Dr. E. R. Harding of the H. J. Heinz Company, who kindly dried these vegetables for us.

The addition of 10% farina to the diet resulted in an increase of 14 mg. calcium; oatmeal, 36 mg., and Pablum, 209 mg. (table 5).

The effect of feeding human diets was studied. A diet for a 17-year-old child (see protocol) with the milk omitted was dried. It should be noted that this diet is such as might be consumed by many people who are not fond of milk. This dried material was used to replace the starch of the low

TABLE 4

Effect of adding 0.115% calcium as $CaCO_3$, as spinach and as $CaCO_3$ plus oxalic acid

MATERIAL ADDED	EXTRA Ca RETAINED	EXTRA P RETAINED
	mg.	*mg.*
$CaCO_3$	306	185
Dried spinach	—81	—82
$CaCO_3$ plus oxalic acid	141	61

TABLE 5

Effect of adding 10% farina, oatmeal and Pablum on Ca retention

MATERIAL ADDED	EXTRA Ca RETAINED	EXTRA P RETAINED
	mg.	*mg.*
Farina	14	43
Oatmeal	36	25
Pablum	209	119

TABLE 6

Effect of human low milk diet and high milk diet on Ca and P retention

DIET	EXTRA Ca RETAINED	RETENTION EXTRA Ca FED	EXTRA P RETAINED	RATIO OF EXTRA $\frac{Ca}{P}$ RETAINED
	mg.	%	*mg.*	
Low milk	50	62.7	20	2.5
High milk	215	88.0	102	2.1

calcium diet, so that each gram of the final mixture contained the equivalent of 1 gm. wet weight of the human diet. In another series of animals, the diet was constructed so that each gram contained the equivalent of 1 gm. wet weight of the 17-year-old child's milkless diet plus the appropriate amount of whole milk which would be present if 1 quart of milk (40 ounces) had been added to the child's milkless diet. In these two experiments, any extra retention of calcium and

phosphorus over the control animals is due to the consumption of the milkless diet in the one case and the same diet plus 1 quart of milk daily in the other case. The results given in table 6 show that not only is the average human diet without milk not a good source of calcium, but the percentage of the calcium retained is less than in the same diet with added milk.

SUMMARY

Studies have been made of the retention in the rat's body of calcium fed in various forms.

When calcium was added to a low calcium but otherwise normal diet, including cod liver oil, in the form of calcium carbonate, calcium chloride, dicalcium phosphate, calcium gluconate, calcium lactate, whole milk powder, casec and Pablum, the percentage of the added calcium retained was essentially the same in each instance.

With an adequate phosphorus and low calcium diet, the retention of calcium and phosphorus increases approximately in the ratio of 2:1 when extra calcium retention is brought about by the addition of calcium to the diet. In other words, in a diet adequate in phosphorus, the amount of phosphorus retained is dependent on the amount of calcium retained.

The addition of calcium in the form of large amounts of spinach to a low calcium diet resulted in a reduction rather than an increase in the body calcium. This effect is probably due in part to the oxalic acid content of the spinach.

The increase in the body calcium produced by the addition of different cereals has been studied.

The inadequacy of an average human diet low in milk, as a source of calcium, has been biologically demonstrated. Not only is the calcium retention on this diet low but the percentage of the calcium retained is less than in the same diet with added milk.

LITERATURE CITED

LEITCH, I. 1937 The determination of the calcium requirements of man. Nutrition Abstr. and Rev., vol. 6, p. 553.

SHERMAN, H. C. 1932 Chemistry of food and nutrition, New York, 4th ed., p. 512.

TISDALL, F. F., T. G. H. DRAKE, P. SUMMERFELDT AND S. H. JACKSON 1937 The comparative value of spinach and tomatoes in the child's diet. J. Pediatrics, vol. 11, p. 374.

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